SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

REPORT DOCUMENTATION PAGE

READ INSTRUCTIONS BEFORE COMPLETING FORM

1. REPORT NUMBER: 3	2. GOVT ACCESSION NO.
3. RECIPIENT'S CATALOG NUMBER	
4. TITLE (and Subtitle): Intrinsic Cholinergic Mechanisms Regulating Cerebral Blood Flow as a Target for Organophosphate ACtion	5. TYPE OF REPORT & PERIOD COVERED: Annual 9/30/86 - 9/29/87
	6. PERFORMING ORG. REPORT NUMBER
7. AUTHOR(s): Donald J. Reis and David A. Ruggiero	8. CONTRACT OR GRANT NUMBER(s): DAMD17-84-C-4185
9. PERFORMING ORGANIZATION NAME AND ADDRESS: Cornell University Medical College, 1300 York Avenue, New York, NY 10021	10. PROGRAM ELEMENT, PROJECT, TASK AREA & WORK UNIT NUMBERS: 61102A, 3M161102BS11 AA 067
11. CONTROLLING OFFICE NAME AND ADDRESS: U.S. Army Medical Research & Development Command, Fort Detrick, Frederick, MD 21701-5012	12. REPORT DATE: October 1988
	13. NUMBER OF PAGES
14. MONITORING AGENCY NAME & ADDRESS(if different from Controlling Office)	15. SECURITY CLASS. (of this report): Unclassified
	15a. DECLASSIFICATION/DOWNGRADING SCHEDULE

16. DISTRIBUTION STATEMENT (of this Report)

Approved for public release; distribution unlimited

1990

17. DISTRIBUTION STATEMENT (of the abstract entered in Block 20, if different from Report)

18. SUPPLEMENTARY NOTES

19. KEY WORDS (Continue on reverse side if necessary and identify by block number)

acetylcholine, choline acetyltransferase, adrenergic neurons, central neural pathways

20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

To investigate acetylcholine's (ACh's) role in cardiovascular and cerebrovascular regulation, neuroanatomic substrates were first defined using immunocytochemical mapping from choline acetyltransferase (ChAT), the ACh-synthesizing enzyme. ChAT-immunoreactive cell bodies and processes were localized to autonomic or limbic nuclei throughout the neuraxis and close appositions with brainstem microvessels and ependymal cells. Moreover, they

DD FORM 1 JAN 73 1473 EDITION OF 1 NOV 65 IS OBSOLETE

SECURITY CLASSIFICATION OF THIS PAGE (When Data Entered)

20. (continued)

codistributed with catecholaminergic neurons staining for tyrosine hydroxylase (TH) or phenylethanolamine N-methyltransferase (PNMT), overlapped structures rich in muscarinic and nicotinic receptors, and expressed autonomic, neuroendocrine or behavioral responsivity to central cholinergic stimulation. Our findings indicate that: (1) cholinergic agents act at multiple sites in the CNS and with topographic specificity; (2) neuroanatomic substrates for cerebrovascular innervation modify microvascular blood flow; (3) cholinergic processing attributed to the locus ceruleus, paraventricular n. and supraoptic n. is mediated polysynaptically or by synapses on processes extending into adjacent cholinoreceptor fields; and (4) anatomical substrates for cholinergic regulation of autonomic reflexes and sympathoexcitation may be mediated by bulbospinal neurons. A rich plexus of varicose fibers overlapping the C1 area of RVL, which provides the excitatory drive for tonic sympathetic discharge, may form the anatomical basis for the increases in sympathetic nerve activity provoked by systemic or central administration of ACh.

AD _____

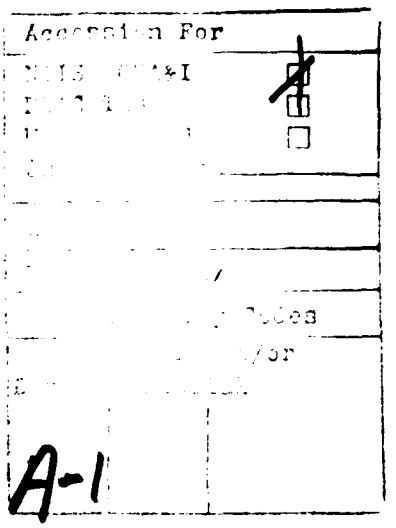
REPORT #3

INTRINSIC CHOLINERGIC MECHANISMS REGULATING CEREBRAL BLOOD FLOW AS A TARGET FOR ORGANOPHOSPHATE ACTION

ANNUAL REPORT

Donald J. Reis and David A. Ruggiero

October 1988



Supported by

U.S. ARMY MEDICAL RESEARCH AND DEVELOPMENT COMMAND
Fort Detrick, Frederick, Maryland 21701-5012

Contract No. DAMD17-84-C-4185

Cornell University Medical College
New York, New York 10021

SUMMARY

In an effort to investigate the role of acetylcholine (ACh) in cardiovascular and cerebrovascular regulation, the groundwork was established by defining neuroanatomic substrates. Cholinergic-autonomic pathways were immunocytochemically mapped using a monoclonal antiserum against choline acetyltransferase (ChAT), the enzyme synthesizing ACH. Central autonomic substructures were defined as areas receiving primary or potentially higher-order visceral afferents, or those with efferent projections to spinal preganglionic neurons of the intermediolateral cell columns (IML).

ChAT-immunoreactive cell bodies and processes were localized to autonomic or limbic nuclei throughout the neuraxis and close appositions with brainstem microvessels and ependymal cells and codistributed with catecholaminergic neurons staining for tyrosine hydroxylase (TH) or phenylethanolamine N-methyltransferase (PNMT). Cholinergic perikarya and putative terminal fields overlapped structures which are rich in muscarinic and nicotinic receptors and express autonomic, neuroendocrine or behavioral responsivity to central cholinergic stimulation (posterior hypothalamic nucleus, fastigial deep cerebellar nucleus, nucleus tractus solitarii (NTS), nucleus reticularis rostroventrolateralis (RVL).

Overall, 1) these data support the concept that cholinergic agents act at multiple sites in the CNS and with topographic specificity. 2) Close appositions were observed between ChAT-positive punctate varicosities and vascular endothelia and ependymal cells lining the floor of the fourth ventricle and the ventral medullary surface, suggest neuroanatomic substrates for cerebrovascular innervation modifying microvascular blood flow. 3) The absence of immunoreactive elements in the locus ceruleus, paraventricular nucleus and supraoptic nucleus was unexpected and suggests that cholinergic processing attributed to these nuclei is mediated polysynaptically or by synapses on processes extending into adjacent cholinoreceptor fields. 4) Putative cholinergic terminals overlapping sites which relay primary (NTS) or higher-order visceral afferents suggest anatomical substrates for cholinergic regulation of autonomic reflexes. 5) ChAT-immunoreactive terminals in areas where cells project to the IML support the view that central cholinergic stimulation provoking sympathoexcitation may be mediated by bulbospinal neurons. A rich plexus of varicose fibers overlapping the C1 area of RVL, which provides the excitatory drive for tonic sympathetic discharge may form the anatomical basis for the increases in sympathetic nerve activity provoked by systemic or central adminstration of cholinergic ACh.

FOREWORD

Acetylcholine (ACh) contributes to the central regulation of autonomic function and intrinsic neurogenic control of the cerebral circulation. Central cholinergic neurons influence multiple autonomic, cerebrovascular and behavioral processes including cardiorespiratory function (Willette et al., 1987). Recent studies have focused on AC as a neurotransmitter regulating microvascular blood flow (Arneric et al., 1988) and on changes in central cholinergic activity as factors in the expression and maintenance of hypertension (Brezenoff and Giuliano, 1982; Giuliano and Brezenoff, 1987; Trimarchi and Buccafusco, 1987). Our recent studies have, in fact, implicated intrinsic cholinergic mechanisms in brain in mediating cerebrovascular provoked by cerebellar stimulation (Reis and Iadecola, 1989): a) in CNS, ChAT and ACh activity and muscarinic receptors were identified in capillary endothelia (Arneric et al., 1988), and b) systemic administration of the antimuscarinic agents, e.g. atropine, abolish the cerebrovasodilation provoked by stimulating the fastigial nucleus in the cerebellum (Nakai et al., 1982; Reis and Iadecola, 1989).

Despite the abundance of functional studies, the sites of action of cholinergic agents and the anatomical pathways subserving cholinergic-autonomic and cerebrovascular regulation are largely unknown. Notably, cholinergic neurons have not been clearly localized to areas known to play a role in central autonomic function. While neurons staining for the cholinergic degradative enzyme acetylcholinesterase (AChE) have been detected in the brainstem reticular formation and raphe (Palkovits and Jacobowitz, 1974; Hoover et al., 1978; Butcher and Woolf, 1984), few were immunoreactive to the ACh-synthesizing enzyme, choline acetyltransferase (ChAT) or localized to physiologically identified areas known to play a role in central autonomic function. Moreover, AChE is not a specific marker for cholinergic cell bodies, being present in noncholinergic (Butcher et al., 1975; Eckenstein and Sofroniew, 1983; Satoh et al., 1983) as well as nonneuronal (Carvalheira and Pearse, 1967) structures, including capillaries and endothelial cells. Finally, the scope of most previous anatomical mapping studies of cholinergic neurons was limited to forebrain and extrapyramidal nuclei concerned with behavioral (memory storage and affective disorders), olfactory or somatomotor functions, providing little information specific to regulation of activities or cerebral blood flow (Sofroniew et al., 1985; Mesulam et al., 1983a,b). The anatomical substrates whereby intrinsic cholinergic networks in brain suspend autoregulation and thereby profoundly modify cerebral blood flow are unknown.

In the present study we have used a monoclonal antibody against ChAT to identify sites in the CNS that may be involved in cholinergic autonomic control and the intrinsic neurogenic control of the cerebral circulation. Our emphasis is on central cholinergic regulation of the circulation. Immunocytochemical studies were designed to map the anatomical substrates of cholinergic-autonomic control by determining whether: 1) cholinergic perikarya are present in traditionally-defined autonomic nuclei; 2) processes are labeled for ChAT in areas of brain which are associated with cholinergically-mediated changes in autonomic function and 3) an anatomical basis exists for cholinergic regulation of AP and cerebral blood flow by neurons such as those in fastigial nucleus and parabrachial complex, the locus ceruleus in the C1 area of the rostral ventrolateral medulla.

In conducting the research described in this report, the investigators adhered to the "Guide for the Care and Use of Laboratory Animals," prepared by the Committee on Care and Use of Laboratory Animals of the Institute of Laboratory Animal Resources, National Research Council (DHEW Publication No. (NIH) 78-23, Revised 1978).

TABLE OF CONTENTS

LIST OF ILLUSTRATIONS

REPORT

METHODS

Experiments were conducted in 25 adult male Sprague-Dawley rats (250 - 400 g). To enhance cell staining ten animals (anesthetized with halothane (2% in 100% O_2) were injected stereotaxically into the right lateral ventricle with colchicine (Sigma; 150 µg in 10 µl distilled water) over 50-60 minutes and allowed to survive for 24 hours.

Animals were anesthetized with Nembutal (50 mg/kg, i.p.) and perfused transcardially with normal saline for 30 seconds followed by 500 ml of 4% (w/v) paraformaldehyde in 0.1 M phosphate buffer (pH 7.3). Frozen 30 µm sections were cut transversely or sagittally on a sliding microtome; alternate sections through the medulla or entire brainstem were processed immunocytochemically for ChAT by a modification of the peroxidase-antiperoxidase technique of Sternberger (1979). Monoclonal antibodies specific to ChAT of porcine brain origin and produced in rat-mouse hybridomas were obtained from Boehringer-Mannheim. In control studies, immunocytochemical specificity was tested by omitting the primary antibody.
In several animals adjacent sections were incubated with primary antisera to ChAT, TH or PNMT. Antibodies to TH and PNMT were produced in rabbits against enzymes of bovine adrenal origin and localized immunocytochemically based on procedures described previously (Park et al., 1982; Joh and Ross, 1983; Ruggiero et al., 1985).

Sections were mounted on gelatin-coated, dehydrated through graded alcohols, cleared in xylene and coverslipped with Histoclad. Section outlines and gross nuclear borders were drawn from adjacent Nissl-stained sections using an overhead Bausch and Lomb projector. The total number of immunoreactive perikarya on each section was mapped under brightfield illumination with the aid of a camera lucida attached to a Leitz microscope. Labeled processes (dendrites, axons and bouton-like varicosities) were drawn under darkfield illumination. The distributions of cholinergic perikarya and fine punctate varicosities were compared to the locations of a) catecholaminergic neurons immunostained on alternate sections, and b) perikarya labeled with wheat germ agglutinin--horseradish peroxidase conjugate (WGA-HRP) or fluorescent tracers (e.g., fluorogold) injected into thoraco-lumbar or cervical segments of spinal cord (most axoplasmic transport materal was available from previous or concomitant (Cravo et al., 1988) studies). Methods used to demonstrate WGA-HRP reaction product were identical to those described previously (Ruggiero et al., 1987).

RESULTS

Previously unidentified cholinergic perikarya, dendrites and punctate varicosities resembling terminal boutons were immunoreactive for ChAT in areas which are known to influence autonomic function. In several areas, a striking correspondence was noted between terminals labeled for ChAT, and both catecholaminergic (TH and PNMT-immunoreactive) and bulbospinal projection cells backfilled with WGA-HRP. On some sections and under high power magnification, terminal fields were traced to their cells of origin. The major trajectories of the more prominent local cholinergic pathways are described. However, in most cases neither their directionality nor distal terminal end stations could be ascertained on normal immunocytochemically-processed material.

HINDBRAIN

In the hindbrain cells and processes were labeled for ChAT in the ventrrolateral medulla, nucleus ambiguus, nucleus solitarius-dorsal motor complex, lateral tegmental field, raphe and paramedian reticular formation, periventricular gray, A5 area and parabrachial complex. The camera lucida drawing of Figure 1 illustrates on sagittal sections ChAT-

immunoreactive cell bodies in autonomic nuclei of the medulla oblongata.

Ventral medulla/nucleus ambiguus: (a) *Ventrolateral medulla.* The majority of ChAT-immunoreactive cells formed a small compact cluster partly overlapping the medial aspect of the rostral ventrolateral reticular nucleus (RVL) (Fig. 1,2). Neurons in this discrete cholinergic subnucleus lay close to the ventral medullary surface and were bordered dorsally and dorsolaterally by adrenergic neurons of the C1 area staining immunocytochemically for the catecholaminergic enzymes TH and PNMT. Cholinergic cell bodies in the medial aspect of the RVL were variable in size (16 μm - 30 μm), shape (fusiform, ovoid and multipolar) and orientation and were slightly smaller than the average long diameter of the C1 catecholaminergic cell bodies labeled for TH and PNMT. Those lying nearest the ventral surface were oriented mediolaterally (horizontally or diagonally), as were most catecholaminergic neurons of the C1 area.

An especially dense thicket of immunoreactive fibers (in part derived from dendrites of the nucleus ambiguus, NA) occurred in the dorsal aspect (apex) of the RVL directly underlying the compact and semicompact divisions of the NA and overlapped bulbospinal neurons backfilled from the cervical cord. Dendrites of ambigual neurons passed ventrally and ventromedially into the RVL (Fig.2). The origins of varicosities in the RVL and lining the ventral medullary surface could not be determined. The trajectories of other ChAT immunopositive processes extended medially between the RVL and the ventral medullary raphe, ventrally along the ventral medullary surface and dorsally into the overlying reticular formation.

b) *Nucleus ambiguus.* Neurons were labeled for ChAT throughout the NA and formed an ovoidal column overlying the nucleus RVL and contributing to the retroambigual portion of the nucleus reticularis caudoventrolateralis (CVL) (Ruggiero et al., 1989, 1990) (Figs. 1 and 2). Cholinergic neurons in the NA comprised several morphologically different subtypes. Proceeding caudally, they demonstrated a progressive increase in long diameter and a decrease in packing density.

Nucleus tractus solitarii: Perikarya in the NTS were labeled moderately to lightly, albeit inconsistently. Labeled cell bodies in the NTS were, moreover, well organized, restricted to specific subnuclei, and consistently localized to the same loci. The majority of cholinergic perikarya occurred in two principal locations: (1) at the junction of the rostral and intermediate thirds of the nucleus (Figs. 3d) and (2) caudally in the ventral subnucleus and the commissural nucleus of the vagus (CNX) (Figs. 1; 3a-c).

In the intermediate third of the NTS a discrete collection of round to ovoid, small perikarya (8-10 μm in diameter) lay medially and dorsomedially to the solitary tract (Fig. 3d). Most cells were located in the intermediate subnucleus and adjacent portions of the medial subnucleus. When viewed under both bright- and dark-field illumination, labeled cells were found to be admixed with immunoreactive punctate varicosities. A second subgroup of cholinergic cell bodies was seen in the ventral NTS (Fig. 3a-c) and extended from the caudal pole of the area postrema into the lateral aspect of CNX. Most cells were situated ventrally and medially to the solitary tract and immediately laterally to the dorsal motor nucleus; some extended laterally and ventrolaterally into an area of the medullary reticular formation, interposed between the dorsal motor nucleus, the ventrolateral division of NTS and the ventral parasolitary region. A third group of lightly to intensely labeled cell bodies was distinguished in the medial aspect (A_2 area) of the CNX, dorsal to the dorsal motor nucleus (Figs. 3).

Axons of passage and punctate varicosities also were labeled in the NTS and extended from the rostral pole of the nucleus to the CNX (Fig. 3a-f). The most prominent fields of putative terminals were localized to the rostral two thirds of the nucleus.

Periventricular gray: Perikarya, presumptive terminals and axons in passage were labeled throughout the brainstem periventricular gray (PVG). ChAT immunoreactive perikarya surrounded the central canal, lined the floor of the fourth ventricle or were concentrated in the dorsal tegmental (DTN) and laterodorsal tegmental (LDT; group Ch6) nuclei and neighboring substructures (Figure 4).

In the closed medulla, clusters of small (8-12 μm), ovoid and pear-shaped perikarya surrounded the perimeter of the central canal. In the rostral medulla, and in increasing numbers throughout the caudal and middle pons, labeled cells were clustered or scattered along the floor of the fourth ventricle. In the medulla, the majority of cell bodies were located medially in the PVG and lay dorsally to the medial vestibular nucleus, nucleus prepositus or medial longitudinal fasciculus (MLF). Most cells were small, ovoid to pear-shaped or spindly and ranged in long diameter from 8 to 20μm.

ChAT-labeled axons also ascended or descended longitudinally within the periventricular gray. Other processes of periventricular cells extended 1) tangentially, in parallel with or perpendicularly to the floor of the fourth ventricle, 2) horizontally across the midline and 3) ventrally into the dorsal raphe or paramedian reticular formation. Immunoreactive processes appeared to contact the stratum subependymale and also surrounded the walls of periventricular microvessels. Similar contacts were observed on sections immunostained for the catecholamine synthesizing enzymes TH and PNMT (Ruggiero et al., 1985). At the isthmus of the pons ChAT-positive cell bodies appeared in the LDT and were variable in shape (multigonal and triangular to fusiform) and approximately 20 μm in long diameter. The major axes of a large proportion of these cell bodies and their principal dendritic arbors were oriented mediolaterally (diagonally or horizontally). Rostrally, the axes of a large number of cells tended to orient diagonally in a dorsomedial to ventrolateral direction. Their processes, like those of other periventricular neurons, coursed dorsally and contacted the subependymal layer of the fourth ventricle. Other fibers coursed dorsolaterally into the lateral parabrachial nucleus, ventrolaterally (at rostral levels) into the medial parabrachial nucleus and contiguous portions of the diffuse division of PPN, and ventromedially into the paramedian reticular formation adjacent to the nucleus raphe pontis and raphe medianis. Punctate varicosities surrounded cholinergic perikarya of the LDT or extended caudally into a geometrically similar area (a non-cholinergic nucleus) lying adjacent to the LDT and just rostrally to the A6 neurons in LC. A major contingent of fibers, which appeared to originate in the LDT, coursed medially through the DTN toward the nucleus raphe dorsalis (RD), nucleus alpha of the central gray and subnucleus O (Meessen and Olszewski, 1949) where they appeared to terminate.

The LC was virtually unlabeled throughout its extent with the exception of a few scattered axons coursing diagonally through it (Fig. 4a, 5b). The nucleus, as defined cytoarchitectonically on Nissl stained sections and immunocytochemically (on adjacent sections) by its content of noradrenergic (TH-immunolabeled) cell bodies backfilled from the spinal cord, was enveloped by the following fields of punctate processes: ventromedially (and rostrally) by Barrington's nucleus and cholinergic cells of the pericentral rim of the DTN; medially by a thin periventricular strip lining the floor and wall of the fourth ventricle; dorsolaterally by a previously undescribed parabrachial field lining the medial border of the superior cerebellar peduncle; and ventrally (and ventrolaterally) by presumptive cholinergic terminals in the mesencephalic trigeminal nucleus and the noradrenergic nucleus subceruleus (identified by TH-positive and bulbospinal projection neurons). ChAT-labeled fibers in passage admixed with clusters of punctate varicosities resembling terminal boutons were followed rostrally through the mesencephalic periaqueductal gray (PAG).

Raphe: ChAT-immunoreactive perikarya were limited to two nuclei of the medullary

raphe: The nucleus raphe obscurus (RO) and nucleus raphe magnus (RM) (Fig. 1). Most cell bodies in the RO extended from the level of the area postrema to the pontomedullary junction. Perikarya of the RO were mostly fusiform or ovoidal and averaged 18-24 μm in long diameter. In the RM, the majority of cell bodies was labeled in the rostral medulla, primarily at the level of the facial nucleus. In a pattern characteristic of serotonergic-spinal cells, ChAT-immunoreactive neurons (and processes) in the raphe extended laterally and ventrolaterally from the midline and were aligned with the transverse fibers of the trapezoid body. A large number of neurons in the RM were fusiform in shape, oriented horizontally and measured approximately 25 μm in long diameter.

In the caudal pons cholinergic varicosities were adjacent to and partly occupied regions retrogradely labeled from the spinal cord and containing 6-hydroxytryptaminergic and dopa-decarboxylase-positive perikarya (see Jaeger et al., 1984, 1985), including the pontine extension of the RM and contiguous parts of the ventral pontine reticular formation. Conspicuous at mid-pontine levels and extending rostrally were two bilaterally symmetrical elongate condensations of labeled processes in the paramedian reticular function, distributed vertically between the medial lemniscus and the MLF. At this level, immunoreactive processes appeared to overlap lateral aspects of the nucleus raphe pontis (RP) and, to a greater degree, adjacent nonserotonergic portions of the paramedian reticular formation. Especially high concentrations of labeled fibers were recognized ventrally in the superior central nucleus (SCN), where the terminal field expanded laterally and lay dorsally and dorsomedially to the reticulotegmental nucleus (RTN). Some fibers clearly bypassed the SCN and passed ventrally into the RTN. midbrain raphe.

A5 Area Cerebellum and Parabrachial Complex: ChAT-immunoreactivity occurred primarily within processes in the A5 area cerebellum and PBC (see Fig. 4a-d). In the ventrolateral pons, ChAT immunoreactive processes overlapped a region lateral to the facial nucleus and superior olive (A5 area). On alternate tissue sections, neurons in the A5 area were immunolabeled for TH and retrogradely labeled from the spinal cord. In the cerebellum, processes were labeled for ChAT in the deep cerebellar cortex (not illustrated). Prominent fields of bouton-like varicosities were identified in the fastigial nucleus and concentrated rostrally within a region innervated by the spinocerebellar tract. Varicosities and thin axon segments were also distributed throughout adjacent folia of the anterior and posterior cerebellar vermis. In the PBC, small numbers of generally lightly labeled cholinergic perikarya, triangular and ovoid in shape and measuring 8-15 μm in long diameter were identified in the prespinal region of the nucleus of Koelliker-Fuse (KF) and scattered within the external-medial and dorsal-lateral nuclei; some perikarya were also embedded in the superior cerebellar peduncle (SCP). Aggregates of punctate varicosities and linear arrays of fibers representing terminal fields and axons in passage were labeled for ChAT in both the lateral and medial divisions of the PBC. As seen under darkfield optics, presumptive terminals in the PBC formed several clusters that were restricted to specific subnuclei as defined by the Nissl and connectional studies of Fulwiler and Saper (1984) (see Fig. 5a).

FOREBRAIN

Hypothalamus: In general, ChAT-positive perikarya in the hypothalamus were sparse, labeled lightly and inconsistently (even in animals pretreated with colchicine) and were scattered without organization. The exception, a small group of intensely immunopositive multipolar cell bodies, was seen in the rostral lateral hypothalamus, in a prespinal area lying dorsolaterally to the supraoptic nucleus (SON) (Fig. 6d,e). At rostral levels a few smaller and inconsistently-labeled perikarya surrounded the perifornical nucleus.

ChAT-containing axons in the hypothalamus, as seen in transverse and sagittal tissue sections, were cut obliquely or perpendicularly to their long axes, and appeared to travel by at

least three principal pathways: 1) the medial forebrain bundle (MFB), 2) the PVG, and 3) the lenticular fasciculus and ansa lenticularis. Immunoreactive varicosities in the hypothalamus were abundant and limited to discrete substructures extending from the mammillary region to the anterior hypothalamic area. Terminal-like fields appeared as dense to moderately dense or light aggregates of punctate varicosities admixed with networks of both fine and coarse processes. In the mammillary region, fibers were labeled in the lateral (LHN) and posterior (PHN) hypothalamic nuclei and the mammillary body (Fig. 6a). In the caudal LHN (Fig. 6a,b) most of the immunoreactivity occurred in axons in passage. Another more densely-labeled field was localized to the lens-shaped subthalamic nucleus (corpus Luysi), indenting the dorsal aspect of the cerebral peduncle. In the PHN (Fig. 6a) a moderately dense plexus extended from the hypothalamic PVG, dorsally, to the supramammillary commissure, ventrally. Fibers in the PHN were in contiguity with label in the ventromedial aspect of the PVG (and subparafascicular nucleus; SPF); they also lay medially to the fasciculus retroflexus and (further rostrally) to the mammillo-tegmental and mammillo-thalamic tracts and caudally to the dorsal hypothalamic area.

In the mammillary body (Figs. 6a), terminal-like varicosities and fibers in passage were concentrated along a dense strip outlining the dorsal arcuate-shaped border of the medial mammillary nucleus. Moderate densities were concentrated along peripheral aspects of both divisions [medial (MM) and lateral (ML)] of the medial mammillary nucleus and encapsulated the lateral mammillary nucleus (LM).

Bouton-like varicosities were labeled in other hypothalamic substructures extending from the tuberal region to the anterior hypothalamic area, including the lateral (LHN), perifornical (PFN) and dorsal (DHN) hypothalamic nuclei and the zona incerta (ZI). In the LHN (Fig. 6b-e) immunoreactive varicosities were concentrated rostrally and laterally, overlying the supraoptic nucleus and bordering the anterior amygdala. This relatively large field extended laterally into the substantia innominata.

Few or no immunoreactive fibers were seen in the paraventricular (PVN), supraoptic (SON) or anterior (AHN) hypothalamic nuclei. In the mediobasal hypothalamus (MBH), including the ventromedial (VMN), arcuate (Arc) and retrochiasmatic (RCN) nuclei, a diffuse immunoreaction product was present on experimental tissues as well as on control sections incubated solely with the secondary antiserum. The PVN contained virtually no labeled varicosities with the exception of a small number of fibers in passage. The PVN was surrounded by neighboring terminal fields in the ZI (dorsally), the DHN (ventrally), and the PFN (ventrolaterally). The main body of the SON (Fig. 6c-e) was also unlabeled and, as described above, was bordered dorsally and dorsolaterally by a prominent field extending from the lateral hypothalamus into the substantia innominata and anterior amygdala.

In the anterior hypothalamus a labeled field outlined the intrahypothalamic component of the bed nucleus of the stria terminalis (NSTi) (just lateral to the unlabeled AHN) (Fig. 5e). The field in the NST was followed rostrally to the level of the anterior commissure where terminals were admixed with labeled perikarya in both dorsal and ventral divisions of the nucleus (Fig. 6f).

ChAT-immunoreactive varicosities coincided: a) catecholaminergic (TH-immunopositive) neurons in posterior, subparafascicular, dorsal and rostrolateral hypothalamic nuclei, including the ventral subcommissural aspect of the NST, and b) bulbospinal cell bodies backfilled from the cervical or thoracolumbar cord in the above nuclei and PFN (but not NST).

Amygdala: The distributions of cells and processes in the amygdala were, in large part, comparable to previous data (e.g. Hellendall et al., 1986) based on ChAT, and AChE

histochemistry; thus, the most densely labeled aggregates of punctate varicosities were localized to the basolateral nucleus, layer 2 of the nucleus of the olfactory tract (NLOT) and the amygdalohippocampal area in that order (Fig. 6b-e).

DISCUSSION

In this study we have identified potential neuroanatomical substrates of cholinergic neurogenic control of the circulation. Most prior works examined the structure and function of the cholinergic subgroups Ch1-Ch6 of the basal forebrain and extrapyramidal system, with little or no focus on areas of autonomic representation (see Shute and Lewis, 1967; Sofroniew et al., 1982, 1985; Mesulam et al., 1983a,b, 1984). Thus, despite ample physiological, pharmacologic and biochemical evidence for circuits involving cholinergic autonomic neurotransmission in the cerebellum RVL, NTS and hypothalamus, the presence of cholinergic or cholinoceptive elements in these areas was either unknown, not studied in detail or complicated by conflicting data.

Our principal findings are, 1) newly identified cholinergic cell groups in the RVL, NTS, and subjacent areas of reticular formation (e.g. nucleus reticularis dorsalis), raphe, periventricular gray and the parabrachial complex and 2) putative terminal fields in the above areas and other substructures known to be involved in autonomic responses, in particular, changes in AP and HR, as, for example, the fastigial nucleus in cerebellum.

The presence of ChAT in cell bodies of central autonomic nuclei, such as the NTS--the first order relay of primary visceral afferents--implies that its end product, ACh, may act as a neurotransmitter in the neurogenic control of autonomic function. ChAT is synthesized by cholinergic perikarya and transported to presynaptic nerve endings (Tucek, 1985). The significance of different intensities of immunoreaction product in individual neurons is unknown. Low or inconsistent levels, as for example, in some neurons of the solitary complex or hypothalamus, suggest that their detection depends on the specific antiserum or that the levels of enzyme in these neurons lie near or below the sensitivity of our technique (see Benno et al., 1982). It is also conceivable that variations in labeling reflect differences in cell function intraventricularly prior to processing for immunochemistry. Overall, a broader and more complexly organized distribution of bouton-like punctata was labeled for ChAT in central autonomic subnuclei than has been reported previously. Putative terminal fields generally conformed to normal cytoarchitecture and were organized topographically (e.g. rostral one-third of fastigial nucleus) or in some areas (e.g., NTS), viscerotopically. Immunocytochemical data provided anatomical substrates for previous functional observations, and also confirmed and extended findings from biochemical assay and receptor binding studies. They also provided greater resolution differentiating cholinoceptive from cholinergic elements. The use of darkfield optics to view tissues processed with the peroxidase-antiperoxidase technique aided greatly in the recognition of labeled processes. This was probably the single major advantage over studies employing brightfield optics to view cholinoceptive fields.

ANATOMICAL CONSIDERATIONS

HINDBRAIN

Ventral medulla: ChAT-positive cell bodies and clusters of punctate varicosities were identified, for the first time, in the reticular formation of a sympathoexcitatory region in the rostral ventrolateral medulla (Ross et al, 1983). Immunoreactive perikarya did not conform to a discrete nucleus, forming instead a compact cluster lying close to the medullary surface and extending between the nucleus gigantocellularis ventralis, medially and the C1 adrenergic area of the nucleus RVL, laterally. On some sections cholinergic reticular neurons extended

laterally into the nucleus RVL as defined functionally by adrenergic and nonadrenergic bulbospinal neurons and terminal projections of the cardiopulmonary NTS (see Ross et al., 1983, 1984a,b, 1985; Cravo et al., 1988; Ruggiero et al., 1989, 1990).

Cholinergic reticular neurons, as described here, do not correspond to previously reported cell groups (Kimura et al., 1981, 1984; Armstrong et al., 1983; Satoh et al., 1983). Armstrong and coworkers (1983), for example, emphasized that the intensity of ChAT-immunoreactivity in neurons of the medullary reticular formation was only slightly higher than background and equivalent to the level of staining observed on control sections. In our material the immunoreaction product in ventral reticular neurons, while tending to be somewhat less than that in neighboring cell groups (for example, the nucleus ambiguus), was appreciably and consistently higher than background. Optimal staining appeared to be dependent on factors such as tissue fixation, the specific antiserum or lot number of the PAP complex, and emphasizes that differences in immunoreactivity often are dependent upon technical parameters, and do not necessarily reflect variations in enzyme content. Thus, differences in technique as much as individual species variation may account for certain discrepancies between our data and those of others. Discrepancies with Satoh et al. (1983), who described perikarya that were AChE positive, ChAT-negative and similar cytoarchitectonically to catecholamine neurons, may be explained by the fact that the degradative enzyme also can occur postsynaptically on the surfaces of cell bodies receiving cholinergic afferents (Wainer et al., 1984). Possibly related was our discovery of a rich plexus of bouton-like varicosities overlying neurons in the nuclei RVL and CVL. Thus, adjacent sections processed for ChAT, TH or PNMT demonstrate that cholinergic processes overlap adrenergic neurons in the C1 area of RVL and non-adrenergic columns of cells admixed with C1 neurons on directly underlying the compact and loose divisions of NA. Whereas C1 adrenergic neurons in RVL appear to project to spinal preganglionic cell columns in thoracic and lumbosacral cord, non-adrenergic perikarya in RVL and the retroambiguual area of CVL may project preferentially to cervico-thoracic phrenic and intercostal lower motoneurons (Ruggiero et al., 1989). Still unknown is the origin of the cholinergic innervation of RVL, viz., whether it derives from intrinsic neurons or distal cholinergic nuclei which interconnect with the RVL (e.g., raphe, NTS, spinal cord). The presence of cholinoceptive elements in the ventrolateral medulla is supported by histochemical staining for AChE (Palkovits and Jacobowitz, 1974; Simon et al., 1981; Satoh et al., 1983), ^{3}H-QNB binding in tissue homogenates (Kobayashi et al., 1978; Simon et al., 1981) and autoradiographic labeling of nicotinic (Clarke et al., 1985, Swanson et al., 1987) and muscarinic receptors (Ernsberger et al., 1988a,b). Consistent with these findings are biochemical markers of cholinergic neurotransmission in the ventral medulla, including measurements of ChAT activity and ACh content (Kobayashi et al., 1975; Vizi and Palkovits, 1978; Helke et al., 1980a,b; Simon et al., 1981) and Ca^{2+}-dependent, K^{+}-evoked release of ACh from the C1 area (Arneric et al., 1986).

Nucleus tractus solitarii: Cholinergic perikarya and fine punctate varicosities resembling individual terminal fields were detected in the NTS. Prior to this report, there was no evidence that NTS neurons synthesized ACh in either the rat (Armstrong et al., 1983; Houser et al., 1983; Kimura et al., 1984; Wainer et al., 1984; Ichikawa and Hirata, 1986), cat (Kimura et al., 1981) or primate (Mesulam et al., 1983a; 1984). The present results suggest that significant numbers of cells are able to biosynthesize ACh in the NTS. Cells in NTS expressed moderate to relatively low levels of enzyme depending on their location and were organized topographically.

Previously unrecognized clusters of punctate varicosities were also labeled for ChAT in the NTS. These processes resembled terminal fields and were distributed viscerotopically. Individual fields, although contiguous, were very well differentiated by their pattern and density, and tended to overlap NTS substructures as defined by cytoarchitecture or patterns of

connectivity. The highest concentrations of cholinergic afferents were found in aspects of the dorsal, medial and intermediate subnuclei which receive first-order afferents from baro-, chemo- and cardiac receptors and the pulmonary tree (Kalia and Sullivan, 1982; Ciriello, 1983; Kalia and Richter, 1985). That cholinergic afferents to the NTS contact neurotransmitter specific neurons is attributed to their limited distribution to portions of subnuclei surrounding the intermediate one-third of the solitary tract. When compared to the distributions of catecholaminergic perikarya (Armstrong et al., 1982; Kalia et al., 1984, 1985), cholinergic processes overlap noradrenergic neurons in the medial subnucleus of the NTS (which are TH- and DBH-positive and PNMT-negative), adrenergic neurons in the dorsal strip (which are TH-, DBH- and PNMT-positive) and dopaminergic neurons in the ventral NTS and dorsal motor nucleus (which are TH-positive and DBH- and PNMT-negative). Cholinergic elements also appear to coincide with the distribution of several neuropeptides in the NTS.

The presence of cholinergic elements in the NTS was predicted by reports of AChE-positive neurons (Butcher and Woolf, 1984; Willenberg et al., 1985; Mizukawa et al., 1986), as well as moderate concentrations of ACh and the cholinergic enzymes ChAT and AChE (Kobayashi et al., 1975; Helke et al., 1980a,b; Simon et al., 1981; Hodes et al., 1983; Hoover et al., 1985; Ernsberger et al., 1988b). Immunocytochemical evidence for cholinergic terminal fields in the NTS is also consonant with receptor binding data (Wamsley et al., 1984a,b; Kobayashi et al., 1978; Simon et al., 1981; Wamsley et al., 1981, 1984a,b; Hodes et al., 1983; Ernsberger et al., 1988a,b; Rotter et al., 1979).

Raphe and Periventricular Gray: Two discrete cholinergic cell groups were identified for the first time in the nucleus raphe magnus (RM) and the nucleus raphe obscurus (RO). Cells in both areas project to the IML (Tohyama et al., 1979; Loewy, 1981; Loewy and McKellar, 1981). ChAT-positive perikarya were not previously localized to the brainstem raphe of rats or primates (Armstrong et al., 1983; Houser et al., 1983; Satoh et al., 1983; Kimura et al., 1984; Satoh and Fibiger, 1986).

Extensive networks of immunolabeled fibers and punctate varicosities were identified throughout the pontine and medullary raphe. This finding extends the work of Kimura et al. (1981) who demonstrated cholinoceptive elements throughout similar areas of the raphe in the cat. Our data also are consistent with the presence of muscarinic and nicotinic cholinoreceptors (Rotter et al., 1979; Wamsley et al., 1981; 1984a,b; and Willenberg et al., 1985), and biochemical evidence of AChE and ChAT activity, as well as muscarinic binding sites (Simon et al., 1981; Ernsberger et al., 1988a,b).

Our immunocytochemical data also revealed, for the first time, parallel trajectories of cholinergic axons coursing through the pontomedullary raphe and paramedian reticular formation. Their diagonally horizontal organization was similar to the monoaminergic projection pattern established for the C1 and C2 adrenergic-raphe fiber tracts as described in the rat (Granata et al., 1985; Ruggiero et al., 1985, 1989, 1990) and cat (Jones and Friedman, 1983).

Cholinergic cells and processes in the medullary periventricular gray (PVG) were more extensive than described previously (see references cited above). The cell groups in the pontine PVG confirm the previous descriptions of Satoh et al. (1983), Satoh and Fibiger (1986) and others. However, a more complex substructural organization of terminal fields was observed at the pontine isthmus, in the dorsal tegmental nucleus and nucleus raphe dorsalis. In contrast to previous reports (Jones and Beaudet, 1987, cats), neither cholinergic cell bodies nor punctata were identified in the locus ceruleus in the rat (see Functional Considerations). Since the monoclonal antibody used by Jones and Beaudet (1987) was similar to ours, the discrepancy might, in fact, reflect a species difference.

Parabrachial complex and A5 area: ChAT-positive cell bodies in the PBC were sparse, labeled lightly and, with the exception of the nucleus of Koelliker-Fuse, scattered without an apparent organization. Armstrong et al. (1983) noted that, in rats, cholinergic cells in the PPN could be followed as far caudally as the PBC, but did not (in contrast to our finding) extend into it or the nucleus of Koelliker-Fuse. Based on the above report, the majority of labeled cells surround the brachium at the pontomesencephalic junction and coincide with the diffuse and compact divisions of the PPN and not the PBC as defined by Nissl stains or tracer studies (Fulwiler and Saper, 1984).

Cholinoceptive elements were described in the PBC in cats (Kimura et al., 1981) and rats (Kimura et al., 1984) as admixed with or directly overlying cholinergic perikarya. Our immunocytochemical data extend these findings. Immunoreactive processes (putative terminals and networks of axons arborizing in passage) were limited to specific areas of the PBC and, in general, conformed to the following subdivisions of Fulwiler and Saper (1984): dorsal, central, internal and superior subnuclei of the lateral parabrachial nucleus, portions of the medial division and the nucleus of Koelliker-Fuse. Another terminal field was identified in an area of the ventrolateral pons (A5) intermingled with catecholaminergic neurons known to project to the intermediolateral cell column (Loewy et al., 1979a). Our data extend and corroborate reports of intense staining of AChE throughout the complex (Butcher and Woolf, 1984), high and low affinity muscarinic agonist binding sites (Wamsley et al., 1981; 1984b) and nicotinic receptors as demonstrated immunocytochemically (Swanson et al., 1987).

FOREBRAIN

Hypothalamus and Amygdala: Our data agree with the general consensus that cholinergic perikarya are rare in the hypothalamus. The exceptions include cells in the lateral hypothalamus overlying the SON, as reported by Mason et al. (1983), or a previously unrecognized population admixed with labeled processes in the perifornical region, as described by Tago et al. (1987). Our data were inconclusive concerning the presence (Sofroniew et al., 1985; Tago et al., 1987) or absence (Rodriguez-Sierra and Morley, 1985) of cholinergic perikarya in the Arc.

In contrast, immunoreactive processes in the hypothalamus were numerous, well organized and limited to cytoarchitectonically well-defined nuclear boundaries. Prior to this study, remarkably little information was available on the distribution of cholinergic afferents in the hypothalamus of the rat (see Sofroniew et al., 1985). As described below, data in the rat were often inconsistent and contradictory, or incomplete and obtained with techniques providing less resolution than ours. In the present investigation, several previously unknown terminal fields were differentiated in 1) the mammillary body (a dorsal immunopositive strip overlying the medial mammillary nucleus and a shell of processes encircling the perimeter of portions of the medial and lateral mammillary nuclei; 2) the posterior and dorsal hypothalamic nuclei (including a distinct field demarcating the ovoid-shaped subparafascicular nucleus); 3) the rostral lateral hypothalamus, overlying the SON; 4) the perifornical nucleus, and 5) the intrahypothalamic component, contiguous with the rostral paracommissural division of the NST. Cholinergic terminals in the ZI were also well organized and arranged in discrete lamina, similar to catecholamine- and ACTH-immunopositive terminals (see Baker et al., 1986), and provide additional evidence that afferents to this region are compartmentalized and organized topographically.

Cells staining for the degradative enzyme AChE (Palkovitz and Jacobowitz, 1974; Satoh et al. 1983) partially overlapped fields labeled for ChAT (posterior hypothalamus, perifornical nucleus or ZI) or regions negative for ChAT, but harboring dopaminergic perikarya (e.g. PVN). It was our impression that 'cholinoceptive' varicosities (immunoreactive for the specific marker ChAT) were often localized to regions harboring catecholaminergic perikarya and

processes (Baker et al., 1986; Chan-Palay et al., 1984a; Ruggiero et al., 1985).

The specificity of cholinergic loci in the hypothalamus was emphasized by other regions containing few or no immunoreactive varicosities. Unexpectedly, and in contrast to microassay and receptor binding data (Palkovits and Jacobowitz, 1974; Helke et al., 1980a,b; Clarke et al., 1985; Altar and Marien 1988), no varicosities were labeled for ChAT in either PVN or the main body of SON. Discrepancies with biochemical data, therefore, suggest that micropunches demonstrating cholinergic markers in the PVN or SON were contaminated by adjacent terminal fields. Our data also are discrepant with reports of structures labeled for AChE in the SON (Mason et al. 1983). In the latter study terminals were restricted to caudal regions of SON predominated by vasopressinergic cells. In our material, cholinergic processes were localized to an area of the lateral hypothalamus adjoining the substantia innominata, but not including the SON. If, as implied by functional studies, cholinergic neurons interact with vasopressin- or dopamine-containing cells in the PVN or SON, the anatomical substrate thus, would be indirect or involve synapses on dendrites occuring outside these nuclei.

Finally, our work provides direct evidence for cholinergic fiber tracts, long and short, traveling in the medial forebrain bundle (MFB), PVG, ansa lenticularis and lenticular fasciculus. Immunopositive axons in the MFB add to the list of chemically-identified processes in this region as reported by Nieuwenhuys et al. (1982) and may represent descending cholinergic fibers from the basal forebrain or those ascending from the PPN or LDT.

In the amygdala, the distributions of cholinergic processes conformed closely to its cytoarchitecture as described by Turner and Zimmer (1984). Our major focus was the central nucleus (Ce), the principal amygdalofugal outflow to brainstem autonomic nuclei. Our principal findings include: 1) A shell of bouton-like punctata and axons encapsulating the Ce, medially (within AP_2) and laterally (within AP_1); 2) In contrast, immunoreactive processes were sparse in Ce, an ovoid structure equivalent to the lateral division of the central nucleus of McDonald (1982). When compared to Golgi preparations (McDonald, 1982), our data suggest that cholinergic processes probably contact dendrites of neurons in the lateral division of Ce which radiate medially into a cholinoceptive area equivalent to AP_2 (the medial division of Ce).

FUNCTIONAL CONSIDERATIONS

Anatomic Substrates of Cholinergic Autonomic Regulation

Our anatomical data, as summarized in Figures 7 and 8, offer new insights into the potential origins of cholinergic influences on autonomic activity and the mechanisms of ACh-mediated: (1) sympathoexcitation and increases in regional cerebral blood flow and adrenal catecholamine synthesis and release, (2) release of vasopressin, and (3) modulation of the baroreceptor and other central autonomic reflexes. In the brainstem, cholinergic processes overlapped or were in close proximity to 1) sympathoexcitatory structures projecting to the IML and associated with cholinergic activation (Figure 7), and 2) nuclei such as the NTS, RVL or the lateral tegmental field and PBC, which relay primary and higher-order sino-aortic baroreceptor and other visceral afferents from the periphery (Figure 8). The widespread, albeit topographically specific, distribution of processes staining for ChAT and overlapping autonomic hypothalamo-, parabrachial-, raphe-, NTS-, and reticulo-spinal neurons may form the morphologic basis for functional evidence that (1) multiple sites in the CNS play a role in cholinergically-mediated (often muscarinic receptor-activated) changes in autonomic function, and that (2) the actions and direction of change in AP and HR or neuronal firing rate provoked by centrally administered cholinergic agonists are site-specific (see Brezenoff and Giuliano, 1982).

In the forebrain, cholinergic processes were localized to sites where microinjections of ACh or cholinergic agonists elicit pressor responses: the septum, posterior hypothalamic nucleus, supramammillary region and pars medialis of the medial mammillary nucleus (Brezenoff 1972; Buccafusco and Brezenoff, 1978, 1979; Pirola et al., 1987). Cholinergic terminals in these regions also may play a role in the baroreflex: Thus, injections of carbachol or physostigmine into the posterior hypothalamic nucleus, an area densely innervated by ChAT-positive processes potentiate the reflex rise in blood pressure to occlusion of the carotid artery--the so-called carotid arterial occlusion reflex (Brezenoff et al., 1982). However, other networks of cholinergic terminals were also found in the subparafascicular nucleus (overlying the posterior hypothalamic subnucleus), lateral hypothalamus (at all levels although, especially rostrally, overlying the SON), zona incerta and dorsal hypothalamic nucleus (adjacent to the PVN), intrahypothalamic and paracommissural components of the bed nucleus of the stria terminalis and portions of the amygdala and septum. Though not mapped systematically for cholinergic-mediated activity, many of these immunoreactive fields may modulate autonomic reflexes by cholinergic mechanisms, perhaps linked to their outflow to the spinal cord.

The absence of cholinergic processes in the PVN and SON was unexpected in light of numerous reports that these nuclei contain cholinergic markers for ACh (Hoover et al., 1978; Helke et al., 1980a,b; Clarke et al., 1985) undergo decreases in ChAT activity in four-week-old spontaneously hypertensive rats (Helke et al., 1980a) and play a role in ACh-mediated release of vasopressin. Functional data, for example, demonstrate that centrally-acting cholinergic agonists release vasopressin (Kuhn, 1974). Since processes containing ChAT did not overlie magnocellular neurosecretory subnuclei but were associated with adjacent areas of the dorsal and lateral hypothalamus, cholinergic control over paraventricular and supraoptic vasopressin-secreting neurons may be mediated by the following anatomical substrates: 1) polysynaptically, 2) by cholinergic synapses on vasopressinergic dendrites extending into adjacent cholinoceptive fields or 3) by adjacent hypothalamic neurons.

Finally, cholinergic forebrain neurons may mediate a repertoire of autonomic activities, perhaps linked to the hypothalamic defense response. These include cholinergic regulation of gastric function by supraoptic (Zawoiski and Koplovits, 1977) and lateral (Okuma et al., 1983) hypothalamic nuclei, body temperature and salivation concomitant with arousal and behavioral excitability (see Avery and Penn, 1973; Beleslin and Stevanovic-Denic, 1986; Tomic-Beleslin and Beleslin, 1986; Warburton, 1981 for references).

In the cerebellum and pons, several cholinergic anatomic substrates were discovered which likely influence autonomic function. Cholinergic processes were observed in the fastigial nucleus (FN) and cerebellar vermis, and within brainstem nuclei contributing to the neurogenic control of cerebral blood flow (e.g. nucleus RVL and rostral third of the medial cerebellar nucleus). These fields, taken with close appositions of ChAT-immunoreactive fibers with intraparenchymal microvessels, provide neuroanatomic substrates for changes in regional cerebral blood flow provoked by brainstem or cerebellar stimulation. Anatomical data predict that cholinergic projections to the PBC may play a role in central autonomic regulation. Cholinergic processes resembling terminal boutons were localized to the fastigial nucleusin cerebellum and subnuclei of the PBC (the medial nucleus, nucleus of Koelliker-Fuse and the dorsal-lateral subnucleus) associated with increases in AP and HR (Miura and Reis, 1970; Doba and Reis, 1972; Mraovitch et al., 1982), the generation of respiratory rhythm (Bertrand and Hugelin, 1971; Cohen, 1979) and projections to spinal preganglionic neurons and bulbar respiratory centers (Saper and Loewy, 1980; Kalia, 1987). Cholinergic afferents also were distributed to parabrachial substructures which, in turn, project to regions (Fulwiler and Saper, 1984) integrating autonomic, limbic and neuroendocrine function, yet having little or no

cholinergic innervation. It is conceivable that by using these indirect pathways, cholinergic afferents may influence non-innervated (non-cholinoceptive) nuclei polysynaptically. The functional significance of these findings has yet to be explored.

Muscarinic-receptor mediated increases in AP attributed to vasopressin secretion may be mediated by ChAT-immunoreactive processes in the medial parabrachial-subceruleal zone where neurons are inhibited tonically by baroreceptor afferents and excited antidromically from the SON (Kannan et al., 1981). Vasopressin secretion, also enhanced by unloading of baroreceptors (Metoki, 1976) or by noxious stimulation (Mirsky et al., 1954) may be modulated by cholinergic afferent mechanisms in the PBC where baroreceptor and somatosympathetic afferents appear to converge (Stornetta et al., 1989).

Cholinoceptive fields in the PBC (and NTS) also overlapped neurons receiving somatic (spinal) and visceral afferents and projecting to the thalamus and cerebral cortex (Nomura et al., 1979; Saper and Loewy, 1980; Saper, 1982a,b; Ruggiero et al., 1986; Stornetta et al., 1989). Cholinergic projections to PBC were found in part to stem from cells in the pedunculopontine (PPN) and laterodorsal tegmental nuclei (LDT), which have similar projections to the forebrain. These circuits, if confirmed ultrastructurally, would provide an anatomical substrate for the increases in REM sleep (and hypotonia) provoked by injecting carbachol into the dorsal parabrachial area (Gnadt and Pegram, 1986).

The LDT (the pontine micturition center located anteriorly to the locus ceruleus) contains, amongst other neurons, cholinergic perikarya admixed with cholinergic processes (Satoh et al., 1978). LDT projection fields, including the ventrolateral NTS (a vesico-relaxer center) and the lateral tegmental field (LTF; a vesico-constrictor center) (Tokunaga and Kuru, 1959; Loewy et al., 1979b; DeGroat, 1975), contain processes labeled for ChAT (this study) and muscarinic binding sites (Ernsberger et al., 1988a,b). Collectively, these data suggest that central cholinergic pathways also may contribute to neural control of the bladder.

The absence of cholinergic processes in the locus ceruleus (LC) was surprising in light of evidence for muscarinic receptors, hemicholinium-3--high affinity choline uptake sites and biochemical markers for ACh (Palkovits and Jacobowitz, 1974; Helke et al., 1980a,b; Butcher and Woolf, 1984; Kasa, 1986) and the observation that microiontophoresis of ACh or cholinergic agonists into the LC decreases AP and HR (Sved and Feldsten, 1987) and increases neuronal firing rates, apparently by a muscarinic receptor-mediated mechanism (Guyenet and Aghajanian, 1979; Engberg and Svensson, 1980). Our immunocytochemical studies demonstrate that cholinergic processes encapsulate, but do not directly overlap noradrenergic perikarya of the A6 area, and therefore do not establish axosomatic synapses. These data suggest that the cholinergic innervation of the LC may occur via synapses on noradrenergic processes lying adjacent to the nucleus.

In the medulla, cholinergic drugs may act to influence autonomic function at three principal sites: (1) the nucleus of the solitary tract (NTS); (2) lateral tegmental field: the nuclei reticularis dorsalis and parvocellularis, and (3) the rostroventrolateral reticular nucleus (RVL). Cholinergic processes were localized to areas of the NTS receiving cardiopulmonary and chemoreceptor afferents (Kalia and Mesulam, 1980; Kalia and Sullivan, 1982; Ciriello, 1983; Kalia and Richter, 1985; Kalia, 1987) and form one anatomic substrate by which cholinergic agonists may exert a modulatory influence on baroreceptor and respiratory reflexes. Cholinergic afferents to subnuclei of the NTS surrounding the solitary tract and projecting to the RVL may modulate reflexes subserved by this circuit (Ruggiero and Reis, 1988; Ruggiero et al., 1989, 1990), including the maintenance of respiratory rhythm (Millhorn and Eldridge, 1986; Feldman and Ellenberger, 1988), the vasodepressor/bradycardic reflex to carotid sinus stretch (Granata et al., 1985), or release of adrenal catecholamines and vasopressin elicited by either baroreceptor-reflex unloading or electrically stimulating the NTS

(Nakai et al., 1982) or the RVL (Ross et al., 1983, 1984b) (Figure 9).

Cholinergic afferents were also localized to the subnucleus centralis of the NTS and nuclei reticularis parvocellularis and dorsalis. Neurons in both regions project to the "compact" formation of NA (Ross et al., 1985; Ruggiero and Reis, 1988; Ruggiero et al., 1989, 1990) supplying pharyngeal, laryngeal, and bronchial smooth muscles, the thymus and pancreas (Gacek, 1975; McAllen and Spyer, 1978; Yoshida et al. 1980; Bullock and Moore, 1981; Lobera et al., 1981; and Laughton and Powley, 1979; Bieger and Hopkins, 1987), and may play a role in modulating swallowing, gastrointestinal and respiratory reflexes (see Ishikawa et al., 1982). Based on the work of Spenser and Talman (1986), microinjections of ACh or cholinergic agonists limited to the intermediate region of NTS (an area innervated by baroreceptor afferents and receiving especially dense cholinergic innervation) provoke changes in AP and HR simulating the baroreflex: Injections of atropine into the NTS which interfere with central cholinergic function attenuate the bradycardic component of the reflex and elevated AP. These observations are supported by our anatomical findings and suggest that the cholinergic afferents identified in this region of the NTS are the critical elements augmenting the baroreceptor reflex and, thus, lowering AP. These data, moreover, may relate to the earlier observation that injections of indirect cholinergic agonists into the lateral ventricle potentiate the reflex slowing of the heart evoked by peripherally administered vasoconstrictor agents or inhibit the reflex tachycardia induced by the vasodilator sodium nitroprusside (Caputi et al., 1980).

Finally, a new field of cholinergic neurons and bouton-like varicosities was recognized in a caudal area of the LTF--the nucleus reticularis dorsalis (RD)--where muscarinic receptors were heavily labeled by [^{3}H]QNB (Ernsberger et al., 1988a; binding sites in RD are contiguous dorsally with those in NTS on their Figure 1A). The nucleus RD also corresponds to an area where pressor responses are provoked by intraparenchymal microinjections of physostigmine (Kubo and Misu, 1983).

ACh may also contribute to the vasomotor and respiratory-generator functions of an area of the ventrolateral medulla physiologically related to nucleus RD. As observed in this study, a rich plexus of varicose processes immunoreactive for ChAT and admixed with perikarya in the nucleus RVL may form the anatomic basis for the following findings: 1) Activation of cholinergic receptors in the RVL by microinjection or ventral surface application of cholinergic agonists elicits dose-related increases in AP and HR (Willette et al., 1984; Bennaroch et al., 1986; Giuliano et al., 1989), as well as cardiorespiratory-stimulation (Dev and Loeschke, 1979; Fukuda and Loeschke, 1979) and reversal of opioid-induced respiratory depression (Willette et al., 1987): both respiratory and vasomotor responses are antagonized by atropine administered by the same route, indicating involvement of muscarinic receptors. 2) The integrity of brainstem-spinal neurons within the area of RVL projecting to the IML (i.e., the C1 region) is critical for the expression of the muscarinic-mediated pressor response to systemically-administered physostigmine (Giuliano et al., 1989): that is, the response is blocked by a) chemical lesions of the RVL specifically destroying C1 area neurons or b) microinjections of drugs (limited to the C1 area as demonstrated autoradiographically) which interfere with central cholinergic function (for example, muscarinic antagonists or the high affinity choline uptake blocker, hemicholinium-3, which depletes endogenous ACh). Lastly, cholinoceptive elements in the C1 area, as identified immunocytochemically, mediate the elevation in AP via M2 receptors (Giuliano et al., 1989): That is, the pressor response is also blocked by injections into the C1 area of the novel M2-muscarinic antagonist, AFDX-116, but is unaffected by the M1-antagonist pirenzepine, or the antinicotinic agent, hexamethonium. Despite compelling evidence for muscarinic-activated sympathoexcitation (and concomitant hypertension) evoked by vasomotor neurons in the C1 area, the anatomical evidence for cholinergic innervation of tonically-firing sympathoexcitatory neurons or interneurons is still indirect.

Finally, multiple neuroanatomical substrates were identified whereby cholinergic neurons in concert with other neurotransmitters influence autonomic function. The importance of ACh in the central regulation of AP (our main focus) is reflected by altered central cholinergic neurotransmission in hypertension, the pressor action of central cholinergic agonists dependent on the functional integrity of the C1 area of RVL and the widespread distribution of ChAT-immunoreaction product coinciding with brain areas that mediate cardiopulmonary reflexes and (in the case of the RVL) maintain basal levels of sympathetic tone (normotension). Combined functional and biochemical studies suggest that changes in cholinergic or AChE activity in specific brain structures may play a role in the physiological response to stress (Gabriel and Soliman, 1983; Gilad et al., 1985; Dilsaver et al., 1986; Hata et al., 1986) concomitant with increases in corticosterone and, perhaps ultimately, in the expression and maintenance of hypertension (Yamori et al., 1972; Buccafusco and Spector, 1980; Helke et al., 1980a,b; Hershkowitz et al., 1983; Giuliano and Brezenoff, 1987; Trimarchi and Buccafusco, 1987). Animal models of hypertension, for example, demonstrate, depending on the phase of the disease process, significant increases in central cholinergic markers (see above) along with changes in central catecholamines (Saavedra et al., 1978; Saavedra and Alexander, 1983) as well as exaggerated responsivity of AP to centrally acting cholinergic agonists and antagonists (Yamori et al., 1972; Hoffman et al., 1978; Kubo and Tatsumi, 1979; Buccafusco and Spector, 1980; Giuliano and Brezenoff, 1987). Selective increases in cholinergic neurotransmission in hypertension, regardless of the etiology, are perhaps, related to the findings that central cholinergic activation simulates the hypothalamic defense response (Beleslin and Stevanovic-Denic, 1986; Tomic-Beleslin and Beleslin, 1986) and that stress, a factor in the pathogenesis of autonomic dysfunction, is as cited above, also associated with changes in cholinergic parameters.

CONCLUSION

Neuroanatomical data shed light on the dependence of cholinergically- provoked sympathoexcitation upon bulbospinal and central catecholaminergic neurons. Most obvious is the extensive overlap of cholinergic processes and brainstem nuclei projecting to the IML and synthesizing dopamine (A11-A13 cells in hypothalamus), norepinephrine (A2 neurons in NTS; A6 dendrites extending into cholinoceptive fields surrounding the locus ceruleus), epinephrine (C1-C3 areas) and substance P, serotonin or enkephalin (ventral medulla, raphe and lateral wings of the nucleus raphe magnus). The C1 area of the RVL, a tonic vasomotor center and a component of the central cholinergic-sympathoexcitatory mechanism and the hypothalamic defense response (Hilton et al., 1983; Hilton and Smith, 1984), may be the critical link (i.e., a final common pathway) in a chain of neurotransmitters mediating cholinergic-autonomic outflow.

Bulbospinal neurons in C1 mediate the pressor response to central muscarinic activation, and are interconnected locally with regions of the raphe harboring neurons synthesizing substance P and acetylcholine (colocalized with serotonin and enkephalins) (see Andrezik et al., 1981; Ruggiero et al., 1984, 1989, 1990). Still to be established are the ultrastructural relationships of cholinergic and vasomotor neurons, the relevance of neurotransmitters cosynthesized by cholinergic neurons (catecholamines in the dorsal motor nucleus, Manier et al., 1987; substance P and atriopeptin in the pons, Standaert et al., 1986; and enkephalins in spinal cord preganglionic neurons, Kondo et al., 1985) and the sequence of molecular and neurochemical changes associated with cholinergic autonomic mechanisms linked perhaps to the pathophysiology of hypertension.

Finally, cholinergic terminals richly innervate forebrain regions--the hypothalamus, amygdala, septohippocampal and pituitary adrenal axes and the periaqueductal gray--involved in conditioned autonomic responses to stress (Gilad et al., 1985, 1986) or the defense arousal

response (Hilton et al., 1983; Hilton and Smith, 1984). These nuclei, in turn, relay to nuclei in the hindbrain synthesizing catecholamines and neuropeptides and modulating the tonic and reflex control of arterial blood pressure (Ruggiero et al., 1989a,b). Changes in brain ACh and receptor sensitivity occur during stress, a factor in autonomic dysfunction. Consequently, dramatic increases in behavioral excitability linked to central cholinergic stimulation, in the face of genetic determinants and a learned response to emotionally arousing stimuli, may contribute to the etiology of neurogenic hypertension.

LITERATURE CITED

Altar, C.A. and M.R. Marien (1988) [^{3}H]vesamicol binding in brain: autoradiographic distribution, pharmacology, and effects of cholinergic lesions. Synapse 2:486-493.

Andrezik, J.A., V. Chan-Palay and S.L. Palay (1981) The nucleus paragigantocellularis lateralis in the rat. Conformation and cytology. Anat. Embryol. 161:355-371.

Armstrong, D.M., C.A. Ross, V.M. Pickel, T.H. Joh and D.J. Reis (1982) Distribution of dopamine-, noradrenaline-, and adrenaline-containing cell bodies in the rat medulla oblongata: demonstrated by the immunocytochemical localization of catecholamine biosynthetic enzymes. J. Comp. Neurol. 212:173-187.

Armstrong, D.M., C.B. Saper, A.I. Levey, B.H. Wainer and R.D. Terry (1983) Distribution of cholinergic neurons in rat brain: demonstrated by the immunocytochemical localization of choline acetyltransferase. J. Comp. Neurol. 216:53-68.

Arneric, S.P., R. Giuliano, P. Ernsberger, M.D. Underwood and D.J. Reis (1986) Does acetylcholine released within the C1 area of the rostral ventrolateral medulla (RVL) tonically maintain arterial pressure? Fed. Proc. 45: 3422.

Avery, D.D. and P.E. Penn (1973) Effects of intrahypothalamic injections of adrenergic and cholinergic substances on behavioral thermoregulation and associated skin temperature levels in rats. Pharmacol. Biochem. Behav. 1:159-165.

Baker, H., D.A. Ruggiero, S. Alden, M. Anwar and D.J. Reis (1986) Anatomical evidence for interactions between catecholamine and ACTH-containing neurons. Neuroscience 17(2):469-484.

Beleslin, D.B. and K. Stevanovic-Denic (1986) A dose response study of aggressive behavioral effects of intracerebroventricular injections of carbachol in cats. Physiol. Behav. 36:75-78.

Benarroch, E.E., A.R. Granata, D.A. Ruggiero, D.H. Park and D.J. Reis (1986) Neurons of the C1 area mediate cardiovascular responses initiated from the ventral medullary surface. Am. J. Physiol. 250:R932-R945.

Benno, R.H., L.W. Tucker, T.H. Joh and D.J. Reis (1982) Quantitative immunocytochemistry of tyrosine hydroxylase in the rat brain. II. Variations in the amount of tyrosine hydroxylase among individual neurons of the locus ceruleus in relationship to neuronal morphology and topography. Brain Res. 246:237-247.

Bertrand, F. and A. Hugelin (1971) Respiratory synchronizing function of nucleus parabrachialis medialis: pneumotaxic mechanism. J. Neurophysiol. 134:189-207.

Bieger, D. and D.A. Hopkins (1987) Viscerotopic representation of the upper alimentary tract

in the medulla oblongata of the rat: The nucleus ambiguus. J. Comp. Neurol. 262:546-562.

Brezenoff, H.E. (1972) Cardiovascular responses to intrahypothalamic injections of carbachol and certain cholinesterase inhibitors. Neuropharmacology 11:637-644.

Brezenoff, H.E., K. Carney and J.J. Buccafusco (1982) Potentiation of the carotid artery occlusion reflex by a cholinergic system in the posterior hypothalamic nucleus. Life Sci. 30: 391-400.

Brezenoff, H.E. and R. Giuliano (1982) Cardiovascular control by cholinergic mechanisms in the central nervous system. Ann. Rev. Pharmacol. Toxicol. 22:341- 381.

Buccafusco, J.J. and H.E. Brezenoff (1978) The hypertensive responses to injection of physostigmine into the hypothalamus of the unanesthetized rat. Clin. Exp. Hypertension. 1:219-227.

Buccafusco, J.J. and H.E. Brezenoff (1979) Pharmacological study of a cholinergic mechanism within the rat posterior hypothalamic nucleus which mediates a hypertensive response. Brain Res. 165:295-310.

Buccafusco, J.J. and S. Spector (1980) Role of central cholinergic neurons in experimental hypertension. J. Cardiovasc. Pharmacol. 2:347-355.

Bullock, K. and R.Y. Moore (1981) Innervation of the thymus gland by brainstem and spinal cord in mouse and rat. Am. J. Anat. 162:157-166.

Butcher, L.L., K. Talbot and L. Bilezikjian (1975) Acetylcholinesterase neurons in dopamine-containing regions of the brain. J. Neural Transmission 37:127-153.

Butcher, L.L. and Woolf, N.J. (1984) Histochemical distribution of acetylcholinesterase in the central nervous system: clues to the localization of cholinergic neurons. In A. Björklund, T. Hökfelt and M.J. Kuhar (eds.) Handbook of Chemical Neuroanatomy, Vol. 3: Classical Transmitters and Transmitter Receptors in the CNS. Amsterdam: Elsevier Science Publishers, pp. 1-50.

Caputi, A.P., F. Rossi, K. Carney and H.E. Brezenoff (1980) Modulatory effect of brain acetylcholine on reflex-induced bradycardia and tachycardia in conscious rats. J. Pharmacol. Exp. Ther. 215:309-316.

Carvalheira, A.F. and A.G.E. Pearse (1967) Comparative cytochemistry of C cell esterases in the mammalian thyroid-parathyroid complex. Histochemie 8:175-182.

Chan-Palay, V., L. Zaborsky, K. Köhler, M. Goldstein and S.L. Palay (1984) Distribution of tyrosine-hydroxylase immunoreactive neurons in the hypothalamus of rats. J. Comp. Neurol. 227: 467-496.

Ciriello, J. (1983) Brainstem projections of aortic baroreceptor afferent fibers in the rat. Neurosci. Lett. 36:37-42.

Clarke, P.B.S., R.D. Schwartz, S.M. Paul, C.B. Pert and A. Pert (1985) Nicotinic binding in rat brain: autoradiographic comparison of ^{3}H-acetylcholine, [^{3}H]nicotine, and ^{125}I-alpha-bungarotoxin. J. Neurosci. 5:1307-1315.

Cohen, M.I. (1979) Neurogenesis of respiratory rhythm in the mammal. Physiol. Rev. 59:

1105-1173.

Cravo, S.L., D.A. Ruggiero, M. Anwar and D.J. Reis (1988) Quantitative- topographic analysis of adrenergic and non-adrenergic spinal projections of cardiovascular area of RVL. Soc. Neurosci. Abstr. 14(1):328.

DeGroat, W.C. (1975) Nervous control of the urinary bladder of the cat. Brain Res. 87:201-211.

Dev, N.B. and H.H. Loeschcke (1979) Topography of the respiratory and circulatory responses to acetylcholine and nicotine on the ventral surface of the medulla oblongata. Pflügers Arch. 379:19-27.

Dilsaver, S.C., R.M. Snider and N.E. Alessi (1986) Stress induces supersensitivity of a cholinergic system in rats. Biol. Psychiatr. 21:1093-1096.

Doba, N. and D.J. Reis (1972) Changes in regional blood flow and cardiodynamics evoked by electrical stimulation of the fastigial nucleus in cat: similarity to orthostatic reflexes. J. Physiol. 227:729-748.

Eckenstein, F. and M.V. Sofroniew (1983) Identification of central cholinergic neurons containing both choline acetyltransferase and acetylcholinesterase and of central neurons containing only acetylcholinesterase. J. Neurosci. 3:2286-2291.

Engberg, G. and T.H. Svensson (1980) Pharmacological analysis of a cholinergic receptor mediated regulation of brain norepinephrine neurons. J. Neural. Trans. 49:137-150.

Ernsberger, P., V. Arango and D.J. Reis (1988a) A high density of muscarinic receptors in the rostral ventrolateral medulla is revealed by correction for autoradiographic efficiency. Neurosci. Lett. 85:179-186.

Ernsberger, P., S.P. Americ, V. Arango and D.J. Reis (1988b) Quantitative distribution of muscarinic receptors and choline acetyltransferase in rat medulla: examination of transmitter-receptor mismatch. Brain Res. 452:336-344.

Feldman, J.L. and H.H. Ellenberger (1988) Central coordination of respiratory and cardiovascular control in mammals. Ann. Rev. Physiol. 50: 593-606.

Fukuda, Y. and H.H. Loeschcke (1979) A cholinergic mechanism involved in the neuronal excitation by H+ in the respiratory chemosensitive structure of the ventral medulla oblongata of rats in vivo. Pflugers Arch. 379:125-135.

Fulwiler, C.E. and C.B. Saper (1984) Subnuclear organization of the efferent connections of the parabrachial nucleus in the rat. Brain Res. Rev. 7:229-259.

Gabriel, N.N. and K.F.A. Soliman (1983) Effect of stress on the acetylcholinesterase activity of the hypothalamus--pituitary--adrenal axis in the rat. Hormone Res. 17: 43-48.

Gacek, R.R. (1975) Localization of laryngeal motor neurons in the kitten. Laryngoscope 85:1841-1860.

Gilad, G.M., V.H. Gilad and J.M. Rabey (1986) Dopaminergic modulation of the septohippocampal cholinergic system activity under stress. Life Sci. 39:2386-2393.

Gilad, G.M., B.D. Mahon, Y. Finkelstein, B. Koffler and V.H. Gilad (1985) Stress-induced activation of the hippocampal cholinergic system and the pituitary-adrenocortical axis. Brain Res. 347:404-408.

Giuliano, R. and H.E. Brezenoff (1987) Increased central cholinergic activity in rat models of hypertension. J. Cardiovasc. Pharmacol. 10:113-122.

Giuliano, R., D.A. Ruggiero, S. Morrison, P. Ernsberger and D.J. Reis (1989) Cholinergic regulation of arterial pressure by the C1 area of the rostral ventrolateral medulla. J. Neurosci. 9:923-942.

Gnadt, J.W. and G.V. Pegram (1986) Cholinergic brainstem mechanisms of REM sleep in the rat. Brain Res. 384:29-41.

Granata, A.R., D.A. Ruggiero, D.H. Park, T.H. Joh and D.J. Reis (1985) Brainstem area with epinephrine neurons mediates baroreflex vasodepressor responses. Am. J. Physiol. 248(Heart Circ. Physiol. 17):H547-H567.

Guyenet, P.G. and G.K. Aghajanian (1979) ACh, substance P and met-enkephalin in the locus coeruleus: pharmacological evidence for independent sites of action. Eur. J. Pharmacol. 53:319-328.

Hata, T., T. Kita, T. Higashiguchi and S. Ichida (1986) Total acetylcholine content and activities of choline acetyltransferase and acetylcholinesterase in brain and duodenum of SART-stressed (repeated cold-stressed) rat. Jpn. J. Pharmacol. 41:475-485.

Helke, C.J., E.A. Muth and D.M. Jacobowitz (1980a) Changes in central cholinergic neurons in the spontaneously hypertensive rat. Brain Res. 188:425-436.

Helke, C.J., B.D. Sohl and D.M. Jacobowitz (1980b) Choline acetyltransferase activity in discrete brain nuclei of DOCA-Salt hypertensive rats. Brain Res. 193:293-298.

Hellendall, R.P., D.A. Godfrey, C.D. Ross, D.M. Armstrong and J.L. Price (1986) The distribution of choline acetyltransferase in the rat amygdaloid complex and adjacent cortical areas, as determined by quantitative micro-assay and immunohistochemistry. J. Comp. Neurol. 249:486-498.

Hershkowitz, M., S. Eliash and S. Cohen (1983) The muscarinic cholinergic receptors in the posterior hypothalamus of hypertensive and normotensive rats. Eur. J. Pharmacol. 86:229-236.

Hilton, S.M., J.M. Marshall and R.J. Timms (1983) Ventral medullary relay neurons in the pathway from the defense areas of the cat and their effect on blood pressure. J. Physiol. (Lond.) 345:149-166.

Hilton, S.M. and P.R. Smith (1984) Ventral medullary neurons excited from the hypothalamic and mid-brain defense areas. J. Autonom. Nerv. Syst. 11:35-42.

Hodes, Z.I., M.A. Rea, D.L. Felton and M.H. Aprison (1983) Specific binding of the muscarinic antagonist ^{3}H-quinuclidinyl benzylate is not associated with preganglionic motor neurons in the dorsal motor nucleus of the vagus. Neurochem. Res. 8:73-87.

Hoffman, W.E., P.G. Schmid and M.I. Phillips (1978) Central cholinergic and noradrenergic stimulation in spontaneously hypertensive rats. J. Pharmacol. Exp. Ther. 206:644-651.

Hoover, D.B., J.C. Hancock and T.E. DePorter (1985) Effect of vagotomy on cholinergic parameters in nuclei of rat medulla oblongata. Brain Res. Bull. 15:5- 11.

Hoover, D.B., E.A. Muth and D.M. Jacobowitz (1978) A mapping of the distribution of acetylcholine, choline acetyltransferase and acetylcholinesterase in discrete areas of rat brain. Brain Res. Bull. 15(1):295-306.

Houser, C.R., G.D. Crawford, R.P. Barber, P.M. Salvaterra and J.E. Vaughn (1983) Organization of morphological characteristics of cholinergic neurons, an immunocytochemical study with a monoclonal antibody to choline acetyltransferase. Brain Res. 266:97-119.

Ishikawa, T., Y. Osumi, M. Fujiwara and M. Nagata (1982) Possible roles of central cholinergic nicotinic mechanisms in regulation of gastric functions. Eur. J. Pharmacol. 80:331-336.

Jaeger, C.B., D.A. Ruggiero, V.R. Albert, D.H. Park, T.H. Joh and D.J. Reis (1984) Aromatic L-amino acid decarboxylase in the rat brain: immunocytochemical localization in neurons of the brain stem. Neuroscience 11:691-713.

Jaeger, C.B., D.A Ruggiero, V.R. Albert, T.H. Joh and D.J. Reis (1985) Immunocytochemical localization of aromatic L-amino acid decarboxylase in the CNS of the rat. In: A. Björkland, T. Hökfelt and K.J. Kuhar (eds), Handbook of Chemical Neuroanatomy. Amsterdam: Elsevier/North Holland, pp. 387-408.

Joh, T.H. and M.E. Ross (1983) Preparation of catecholamine-synthesizing enzymes as immunogens for immunocytochemistry. In: A.C. Cuello (ed.), Immunocytochemistry. IBRO Handbook Series: Methods in the Neurosciences, John Wiley and Sons, New York, Vol. 3, pp. 121- 138.

Jones, B.E. and A. Beaudet (1987) Distribution of acetylcholine and catecholamine neurons in the cat brainstem: a choline acetyltransferase and tyrosine hydroxylase immunohistochemical study. J. Comp. Neurol. 261:15-32.

Jones, B.E. and L. Friedman (1983) Atlas of catecholamine perikarya, varicosities and pathways in the brainstem of the cat. J. Comp. Neurol. 215:382-396.

Kalia, M.P. (1987) Organization of central control of airways. Ann. Rev. Physiol. 49:595-609.

Kalia, M., K. Fuxe and M. Goldstein (1985) Rat medulla oblongata. II. Dopaminergic, noradrenergic (A1 and A2) and adrenergic neurons, nerve fibers and presumptive terminal processes. J. Comp. Neurol. 233:208-332.

Kalia, M., K. Fuxe, T. Hökfelt, O. Johansson, R. Lang, D. Ganten, C. Cuello and L. Terenius (1984) Distribution of neuropeptide immunoreactive nerve terminals within the subnuclei of the nucleus tractus solitarius of the rat. J. Comp. Neurol. 222:409-444.

Kalia, M. and M.M. Mesulam (1980) Brain stem projections of sensory and motor components of the vagus complex in the cat. II. Laryngeal, tracheobronchial, pulmonary, cardiac and gastrointestinal branches. J. Comp. Neurol. 193:467-508.

Kalia, M. and D. Richter (1985) Morphology of physiologically identified slowly adapting lung stretch receptor afferents stained with intra-axonal horseradish peroxidase in the nucleus of the tractus solitarius of the cat. I. A light microscopic analysis. J. Comp. Neurol.

241:503-520.

Kalia, M. and J.M. Sullivan (1982) Brainstem projections of sensory and motor components of the vagus nerve in the rat. J. Comp. Neurol. 221:248-264.

Kannan, H., K. Yagi and Y. Sawaki (1981) Pontine neurones: electrophysiological evidence of mediating carotid baroreceptor inputs to supraoptic neurones in rats. Exp. Brain Res. 42:362-370.

Kasa, P. (1986) The cholinergic systems in brain and spinal cord. Prog. in Neurobiol. 26: 211-272.

Kimura, H., P.L. McGeer and J.-H. Peng (1984) Choline acetyltransferase- containing neurons in the rat brain. In: A. Björklund, T. Hökfelt and M.J. Kuhar (eds.), Handbook of Chemical Neuroanatomy, Vol. 3: Classical Transmitters and Transmitter Receptors in the CNS, Part II. Amsterdam: Elsevier Science Publishers, pp. 51-67.

Kimura, H., P.L. McGeer, J.-H. Peng and E.G. McGeer (1981) The central cholinergic system studied by choline acetyltransferase immunohistochemistry in the cat. J. Comp. Neurol. 200:151-202.

Kobayashi, R.M. M. Brownstein, J.M. Saavedra and M. Palkovits (1975) Choline acetyltransferase content in discrete regions of the rat brainstem. J. Neurochem. 24:637-640.

Kobayashi, R.M., M. Palkovits, R.E. Hruska, R. Rothschild and H.I. Yamamura (1978) Regional distribution of muscarinic cholinergic receptors in rat brain. Brain Res. 154:13-23.

Kondo, H., H. Kuramoto, B.H. Wainer and N. Yanaihara (1985) Evidence for the coexistence of acetylcholine and enkephalin in the sympathetic preganglionic neurons of rats. Brain Res. 335:309-314.

Kubo, T. and Y. Misu (1983) The nucleus reticularis dorsalis: a region sensitive to physostigmine. Neuropharmacol. 22:1155-1158.

Kubo, T. and M. Tatsumi (1979) Increased pressor response to physostigmine in spontaneously hypertensive rats. Naunyn Schmiedebergs Arch. Pharmacol. 306:81- 83.

Kühn, E.R. (1974) Cholinergic and adrenergic release mechanism for vasopressin in the male rat: a study with injections of neurotransmitters and blocking agents into the third ventricle. Neuroendocrinol. 16:255-264.

Laughton, W. and T.L. Powley (1979) Four central nervous system sites project to the pancreas. Soc. Neurosci. Abstr. 5:46.

Lobera, B., R. Pasaro, S. Gonzalez-Baron and J.M. Delgado-Garcia (1981) A morphological study of ambiguus nucleus motoneurons innervating the laryngeal muscles in the rat and cat. Neurosci. Lett. 23:125-130.

Loewy, A.D. (1981) Raphe pallidus and raphe obscurus projections to the intermediolateral cell column in the rat. Brain Res. 222: 129-133.

Loewy, A.D. and S. McKellar (1981) Serotonergic projections from the ventral medulla to the intermediolateral cell column in the rat. Brain Res. 211:146-152.

Loewy, A.D., S. McKellar and C.P. Saper (1979a) Direct projections from the A5 catecholamine cell group to the intermediolateral cell column. Brain Res. 174: 309-314.

Loewy, A.D., C.B. Saper and R.P. Baker (1979b) Descending projections from the pontine micturition center. Brain Res. 172:533-538.

Manier, M., P. Mouchet and C. Feuerstein (1987) Immunohistochemical evidence for the coexistence of cholinergic and catecholaminergic phenotypes in neurones of the vagal motor nucleus in the adult rat. Neurosci. Lett. 80:141-146.

Mason, W.T., Y.W. Ho, F. Eckenstein and G.I. Hatton (1983) Mapping of cholinergic neurons associated with rat supraoptic nucleus: combined immunocytochemical and histochemical identification. Brain Res. Bull. 11:617-626.

McAllen, R.M. and K.M. Spyer (1978) Two types of vagal preganglionic motoneurons projecting to the heart and lungs. J. Physiol. (Lond.) 282:353-364.

McDonald, A.J. (1982) Cytoarchitecture of the central amygdaloid nucleus of the rat. J. Comp. Neurol. 208:401-418.

Meessen, H. and J. Olszewski (1949) A Cytoarchitectonic Atlas of the Rhombdencephalon of the Rabbit. Basel: Karger.

Mesulam, M.M., E.J. Mufson, A.I. Levey and B.H. Wainer (1983a) Cholinergic innervation of cortex by the basal forebrain: cytochemistry and cortical connections of the septal area, diagonal band nuclei, nucleus basalis (substantia innominata) and hypothalamus in the rhesus monkey. J. Comp. Neurol. 214:170- 197.

Mesulam, M.M., E.J. Mufson, A.I. Levey and B.H. Wainer (1984) Atlas of cholinergic neurons in the forebrain and upper brainstem of the macaque based on monoclonal choline acetyltransferase immunohistochemistry and acetylcholinesterase histochemistry. Neuroscience 12:669-686.

Mesulam, M.M., E.J. Mufson, B.H. Wainer and A.I. Levey (1983b) Central cholinergic pathways in the rat: an overview based on an alternative nomenclature (Ch1-Ch6). Neuroscience 10:1185-1201.

Metoki, T. (1976) Antidromically identified neurohypophyseal neurosecretory neurons: effects of hemorrhage, retransfusion and hyperosmolal stimulation on unit activity in the rat. Brain Res. 107:424-428.

Millhorn, D.E. and F.L. Eldridge (1986) Role of ventrolateral medulla in regulation of respiratory and cardiovascular systems. J. Appl. Physiol. 61:1249-1263.

Mirsky, I.A., Stein, M. and Paulisch, G. (1954) The secretion of an antidiuretic sustance into the circulation of rats exposed to noxious stimuli. J. Neuroendocrinol. 54:491-505.

Miura, M. and D.J. Reis (1970) A blood pressure response from fastigial nucleus and its relay pathway in brainstem. Am. J. Physiol. 219:1330-1336.

Mizukawa, K., P.L. McGeer, H. Tago, J.H. Peng, E.G. McGeer and H. Kimura (1986) The cholinergic system of the human hindbrain studied by choline acetyltransferase immunohistochemistry and acetylcholinesterase histochemistry. Brain Res. 379:39-55.

Mraovitch, S., M. Kumada and D.J. Reis (1982) Role of the nucleus parabrachialis in cardiovascular regulation in cat. Brain Res. 232:57-75.

Nakai, M., Y. Yamane, Y. Umeda and K. Ogino (1982) Vasopressin-induced pressor response elicited by electrical stimulation of solitary nucleus and dorsal motor nucleus of vagus of rat. Brain Res. 251:164-168.

Niewenhuys, R., L.M.G. Geeraedts and J.G. Veening (1982) The medial forebrain bundle of the rat. I. General introduction. Comp. Neurol. 206:49-81.

Nomura, S., N. Mizuno, K. Itoh, K. Matsuda, T. Sugimoto and Y. Nakamura (1979) Localization of parabrachial nucleus neurons projecting to the thalamus or the amygdala in the cat using horseradish peroxidase. Exp. Neurol. 64: 375-385.

Okuma, Y., Y. Osumi, T. Ishikana and M. Nagata (1983) Central noradrenergic-cholinergic interaction in regulation of gastric secretion. Life Sci. 32:1363-1370.

Palkovits, M. and D.M. Jacobowitz (1974) Topographic atlas of catecholamine and acetylcholinesterase-containing neurons in the rat brain. II. Hindbrain (mesencephalon, rhombencephalon). J. Comp. Neurol. 157:29-42.

Park, D.H., E.E. Baetge, B. Kaplan, V.R. Albert, D.J. Reis and T.H. Joh (1982) Different forms of adrenal phenylethanolamine N-methyltransferase: species- specific posttranslational modification. J. Neurochem. 38:410-414.

Pirola, C.J., A. Scheucher, M.S. Balda, S.M. Dabsys, S. Finkielman and V.E. Nahmod (1987) Serotonin mediates cardiovascular responses to acetylcholine, bradykinin, angiotensin II and norepineprine in the lateral septal area of the rat brain. Neuropharmacology 26(6):561-566.

Reis, D.J. and Iadecola, C.: Central neurogenic regulation of cerebral blood flow. In: Neurotransmission and Cerebrovascular Function II, Amsterdam: Elsevier, 1989, pp. 369-390.

Rodriguez-Sierra, J.F. and B.J. Morley (1985) Evidence that cell bodies in the arcuate nucleus of the hypothalamus are not cholinergic. Neuroendocrinol. 41:427- 431.

Ross, C.A., D.A. Ruggiero, T.H. Joh, D.H. Park and D.J. Reis (1983) Adrenaline synthesizing neurons in the rostral ventrolateral medulla: a possible role in tonic vasomotor control. Brain Res. 273:356-361.

Ross, C.A., D.A. Ruggiero, T.H. Joh, D.H. Park and D.J. Reis (1984a) Rostral ventrolateral medulla: selective projections to the thoracic autonomic cell column from the region containing C1 adrenaline neurons. J. Comp. Neurol. 228:168-184.

Ross, C.A., D.A. Ruggiero, D.H. Park, T.H. Joh, A.F. Sved, J. Fernandez-Pardal, J.M. Saavedra, and D.J. Reis (1984b) Tonic vasomotor control by the rostral ventrolateral medulla: Effect of electrical or chemical stimulation of C1 adrenaline containing neurons on arterial pressure, heart rate, and plasma catecholamines and vasopressin. J. Neurosci. 4: 474-494.

Ross, C.A., D.A. Ruggiero and D.J. Reis (1985) Projections from the nucleus tractus solitarii to the rostral ventrolateral medulla. J. Comp. Neurol. 242:511-534.

Rotter, A., N.J.M. Birdsall, P.M. Field and G. Raisman (1979) Muscarinic receptors in the central nervous system of the rat. II. Distribution of binding of ^{3}H-propylbenzilcholine mustard in the midbrain and hindbrain. Brain Res. Rev. 1:167-183.

Ruggiero, D.A., S. Cravo, V. Arango and D.J. Reis (1989a) Central control of the circulation by the rostral ventrolateral reticular nucleus: anatomical substrates. In: Progress in Brain Research, J. Ciriello, C. Polosa and C. Caverson, eds., in press.

Ruggiero, D.A. and D.J. Reis (1988) Neurons containing phenylethanolamine N-methyltransferase: a component of the baroreceptor reflex? In: J.M. Stolk, D.C. U'Prichard and K. Fuxe (eds.), Epinephrine in the Central Nervous System. New York: Oxford Univ. Press, pp. 291-307.

Ruggiero, D.A., S. Mraovitch, A.R. Granata, M. Anwar and D.J. Reis (1987) A role of insular cortex in autonomic function. J. Comp. Neurol. 257: 189-207.

Ruggiero, D.A., C.A. Ross, M. Anwar, D.H. Park, T.H. Joh and D.J. Reis (1985) Distribution of neurons containing phenylethanolamine N-methyltransferase in medulla and hypothalamus of rat. J. Comp. Neurol. 239:127-154.

Ruggiero, D.A., R.L. Stornetta, S.F. Morrison and D.J. Reis (1986b) Spinal projections to brainstem autonomic centers. Soc. Neurosci. Abstr. 12:1156.

Ruggiero, D.A., P. Ernsberger, S.F. Morrison and D.J. Reis (1989b) Regulation of arterial blood pressure by the rostral ventrolateral medulla. In: Foundations of Behavioral Medicine: Cardiovascular Disease. Lawrence Erlbaum Assoc., in press.

Ruggiero, D.A., Ross, C.A., Kumada, M., and Reis, D.J. (1982) Reevaluation of projections from the mesencephalic trigeminal nucleus to the medulla and spinal cord: new projections. A combined retrograde and anterograde horseradish peroxidase study. J. Comp. Neurol. 206: 278-292.

Saavedra, J.M. and N. Alexander (1983) Catecholamines and phenylethanolamine N-methyltransferase in selected brain nuclei and the pineal gland of neurogenically hypertensive rats. Brain Res. 274:388-392.

Saavedra, J.M., H. Grobecker and J. Axelrod (1978) Changes in central catecholaminergic neurons in the spontaneously hypertensive (genetic) rat. Circ. Res. 42: 529-534.

Saper, C.B. and A.D. Loewy (1980) Efferent connections of the parabrachial nucleus in the rat. Brain Res. 197:291-317.

Saper, C.B. (1982a) Convergence of autonomic and limbic connections in the insular cortex of the rat. J. Comp. Neurol. 210:163-173.

Saper, C.B. (1982b) Reciprocal parabrachial-cortical connections in the rat. Brain Res. 197:291-317.

Satoh, K., D.M. Armstrong and H.C. Fibiger (1983) A comparison of the distribution of central cholinergic neurons as demonstrated by acetycholinesterase pharmacohistochemistry and choline acetyltransferase immunohistochemistry. Brain Res. Bull. 11:693-720.

Satoh, K. and K. Fibiger (1986) Cholinergic neurons of the laterodorsal tegmental nucleus: efferent and afferent connections. J. Comp. Neurol. 253:277-302.

Satoh, K., M. Tohyama, T. Sakumoto, K. Yamamoto and M. Shimizu (1978) Descending projections from the nucleus tegmentalis laterodorsalis to the spinal cord; studies by the

horseradish peroxidase method following 6-hydroxy-DOPA administration. Neurosci. Lett. 8:9-15.

Shute, C.C.D. and P.R. Lewis (1967) The ascending cholinergic reticular system: neocortical, olfactory and subcortical projections. Brain 90:497-520.

Simon, J.R., B. Oderfeld-Nowak, D.L. Felton and M.H. Aprison (1981) Distribution of choline acetyltransferase, acetylcholinesterase, muscarinic receptor binding, and choline uptake in discrete areas of the rat medulla oblongata. Neurochem. Res. 6:497-505.

Sofroniew, M.V., P.E. Campbell, A.C. Cuello and F. Eckenstein (1985) Central cholinergic neurons visualized by immunohistochemical detection of choline acetyltransferase. In: G. Paxinos (ed.), The Rat Nervous System, Vol. 1: Forebrain and Midbrain. New York: Academic Press, pp. 471-485.

Sofroniew, M.V., F. Eckenstein, H. Thoenen and A.C. Cuello (1982) Topography of choline acetyltransferase-containing neurons in the forebrain of the rat. Neurosci. Lett. 33:7-12.

Spencer, S.E. and W.T. Talman (1986) Central modulation of gastric pressure by substance P: a comparison with glutamate and acetylcholine. Brain Res. 385:371- 374.

Standaert, D.G., C.B. Saper, D.B. Rye and B.H. Wainer (1986) Colocalization of atriopeptin-like immunoreactivity with choline acetyltransferase- and substance P-like immunoreactivity in the pedunculopontine and laterodorsal tegmental nuclei in the rat. Brain Res. 382:163-168.

Sternberger, L.A. (1979) Immunocytochemistry. Englewood Cliffs, NJ: Prentice-Hall.

Stornetta, R., S.L. Morrison, D.A. Ruggiero and D.J. Reis (1989) Neurons in the rostral ventrolateral medulla mediate the somatic pressor reflex. Am. J. Physiol. 256:R448-462.

Swanson, L.W., D.M. Simmons, P.J. Whiting and J. Lindstrom (1987) Immunohistochemical localization of neuronal nicotinic receptors in the rodent central nervous system. J. Neurosci. 7:3334-3342.

Sved, A.F. and G. Felsten (1987) Stimulation of the locus coeruleus decreases arterial pressure. Brain Res. 414:119-132.

Tago, H., P.L. McGeer, G. Bruce and L.B. Hersh (1987) Distribution of choline acetyltransferase-containing neurons of the hypothalamus. Brain Res. 415:49-62.

Tohyama, M., K. Sakai, M. Touret, D. Salvert and M. Jouvet (1979) Spinal projections from the lower brianstem in the cat as demonstrated by the HRP technique. II. Projections from the dorsolateral pontine tegmentum and raphe nuclei. Brain Res. 176: 215-231.

Tokunaga, S. and M. Kuru (1959) Vesico-constrictor center and vesico-relaxer center in the medulla. Jap. J. Physiol. 9:365-374.

Tomic-Beleslin, N. and D.B. Beleslin (1986) Salivation mediated by central M-2 muscarinic receptors in the cat. Brain Res. Bull. 17:279-281.

Trimarchi, G.R. and J.J. Buccafusco (1987) Changes in regional brain synaptosomal high affinity choline uptake during the development of hypertension in spontaneously hypertensive rats. Neurochem. Res. 12:247-254.

Tucek, S. (1985) Regulation of acetylcholine synthesis in the brain. J. Neurochem. 44:11-24.

Turner, B.H. and J. Zimmer (1984) The architecture and some of the interconnections of the rat's amygdala and lateral periallocortex. J. Comp. Neurol. 227:540-557.

Vizi, S.E. and M. Palkovits (1978) Acetylcholine content in different regions of the rat brain. Brain Res. Bull. 3:93-96.

Wainer, B.H., A.I. Levey, E.J. Mufson and M.M. Mesulam (1984) Cholinergic systems in mammalian brain identified with antibodies against choline acetyltransferase. Neurochem. Int. 6:163-182.

Wamsley, J.K., D.R. Gehlert, W.R. Roeske and H.I. Yamamura (1984a) Muscarinic antagonist binding site heterogeneity as evidenced by autoradiography after direct labeling with ^{3}H-QNB and ^{3}H-pirenzepine. Life Sci. 34:1395-1402.

Wamsley, J.K., M.S. Lewis, W.S. Young III and M.J. Kuhar (1981) Autoradiographic localization of muscarinic cholinergic receptors in rat brainstem. J. Neurosci. 1(2):176-191.

Wamsley, J.K., M.A. Zarbin and M.J. Kuhar (1984b) Distribution of muscarinic cholinergic high and low affinity agonist binding sites: a light microscopic autoradiographic study. Brain Res. Bull. 12(3):233-243.

Warburton, D.M. (1981) Neurochemistry of behavior. Brit. Med. Bull. 37:121-125.

Willenberg, I.M., R. Dermietzel, A.G. Leibstein and M. Effenberger (1985) Mapping of cholinoceptive (nicotinoceptive) neurons in the lower brainstem: with special reference to the ventral surface of the medulla. J. Autonom. Nerv. Sys. 14:287- 298.

Willette, R.N., B.M. Doorley and H.N. Sapru (1987) Activation of cholinergic mechanisms in the medulla oblongata reverse intravenous opioid-induced respiratory depression. J. Pharmacol. Exp. Ther. 240:352-358.

Willette, R.N., S. Punnen, A.J. Krieger and H.N. Sapru (1984) Cardiovascular control by cholinergic mechanisms in the rostral ventrolateral medulla. J. Pharmacol. Exp. Ther. 231:457-463.

Yamori, Y., H. Yamabe, W. DeJong, W. Lovenberg and A. Sjoerdsma (1972) Effect of tissue norepinephrine depletion by 6-hydroxydopamine on blood pressure in spontaneously hypertensive rats. Eur. J. Pharmacol. 17:135-140.

Yoshida, Y., T. Miyazaki, M. Hirano, T. Shin, T. Totoki and T. Kanaseki (1980) Location of motoneurons supplying the cricopharyngeal muscle in the cat studied by means of the horseradish peroxidase method. Neurosci. Lett. 18:1-4.

Zawoiski, E.J. and Koplovits I. (1977) Gastric secretory response of the anesthetized dog after direct chemical stimulation of the supraoptic region. Exp. Neurol. 55:122-132.

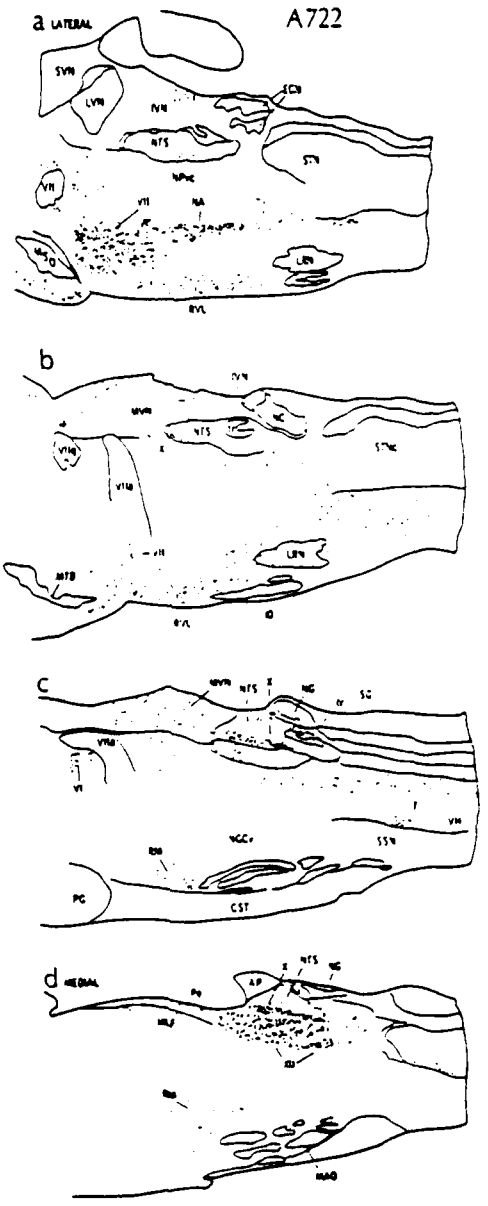


Figure 1. Camera lucida drawings depicting distributions of ChAT-labeled neurons on sagittal sections of the medulla oblongata. Each black dot represents one immunolabeled cell body in a single 30 μm section. Labeled cell bodies were localized to several autonomic substructures including: the NTS, raphe, lateral tegmental field, rostral ventrolateral medulla and periventricular grey.

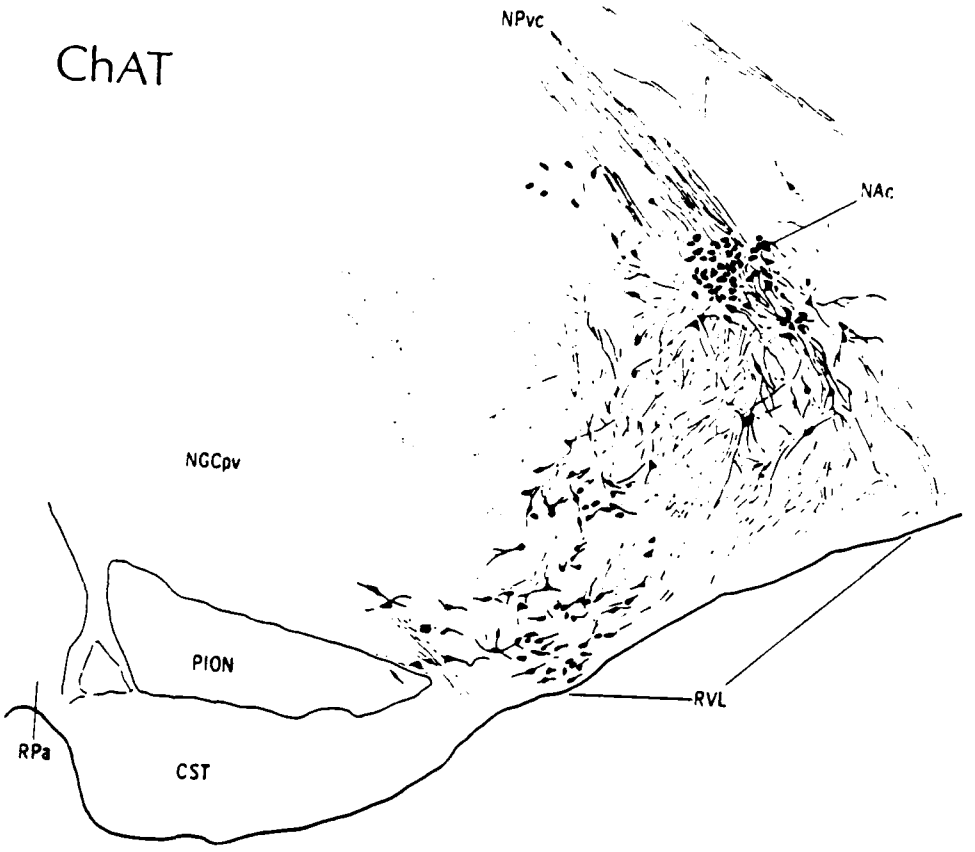


Figure 2. Camera lucida drawings of neurons staining for ChAT in the RVL. ChAT-stained neurons lie in the medial RVL close to the ventral surface, and are smaller and primarily ventromedial to the catecholaminergic (TH-labeled) neurons of the C1 area. Dendrites of neurons of the nucleus ambiguus (NA) extend into the RVL. Very fine immunoreactive punctate processes resembling terminals (as confirmed by electron microscopy, Milner et al., in press) are also present in the RVL.

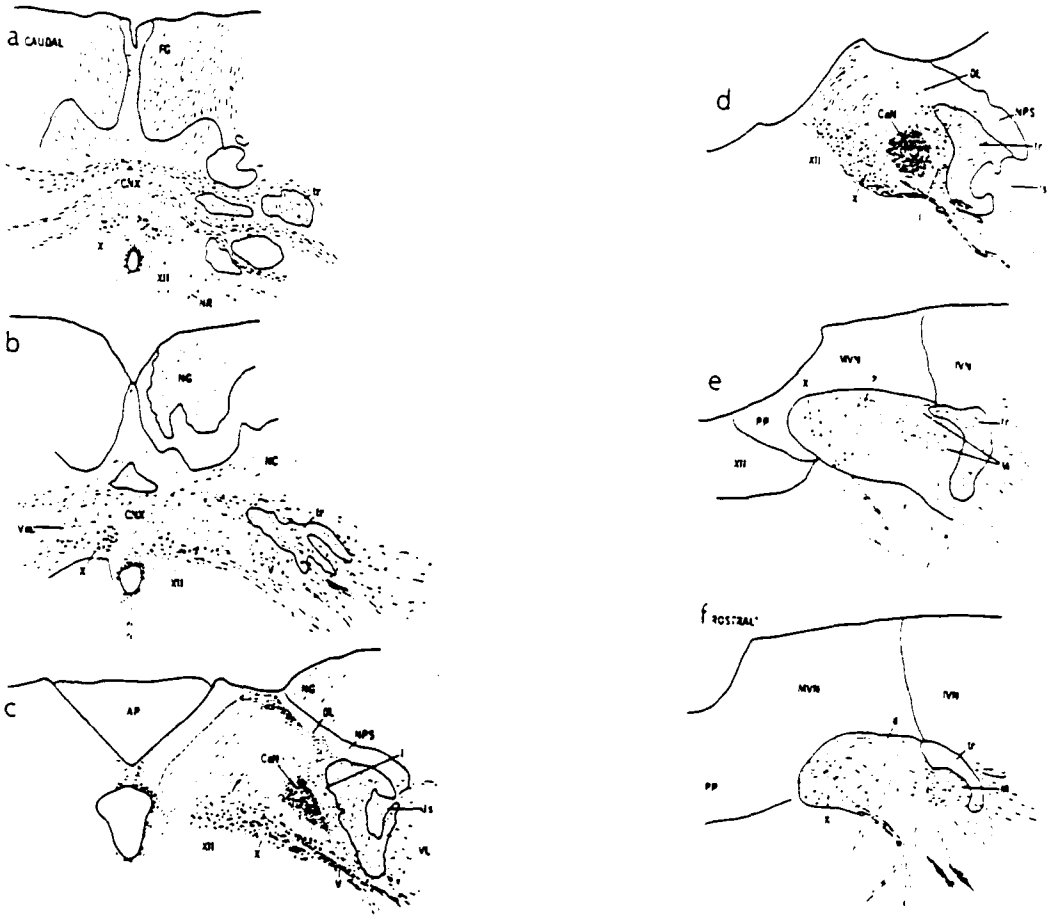


Figure 3. **Camera lucida drawings depicting ChAT-immunoreactivity at commissural (a,b) intermediate (c,d) and rostral (e,f) levels of the nucleus tractus solitarii (NTS). Perikarya (*filled circles*) were labeled in commisural (a), ventral (b,c), medial and intermediate (d-f) subnuclei. Labeled terminals (fine punctate varicosities) are limited to discrete fields of the NTS within dorsal, intermediate and medial subnuclei. On the basis of painstaking analysis of serially sectioned tissues it was our impression that processes in the NTS are derived from both extrinsic and local neurons.**

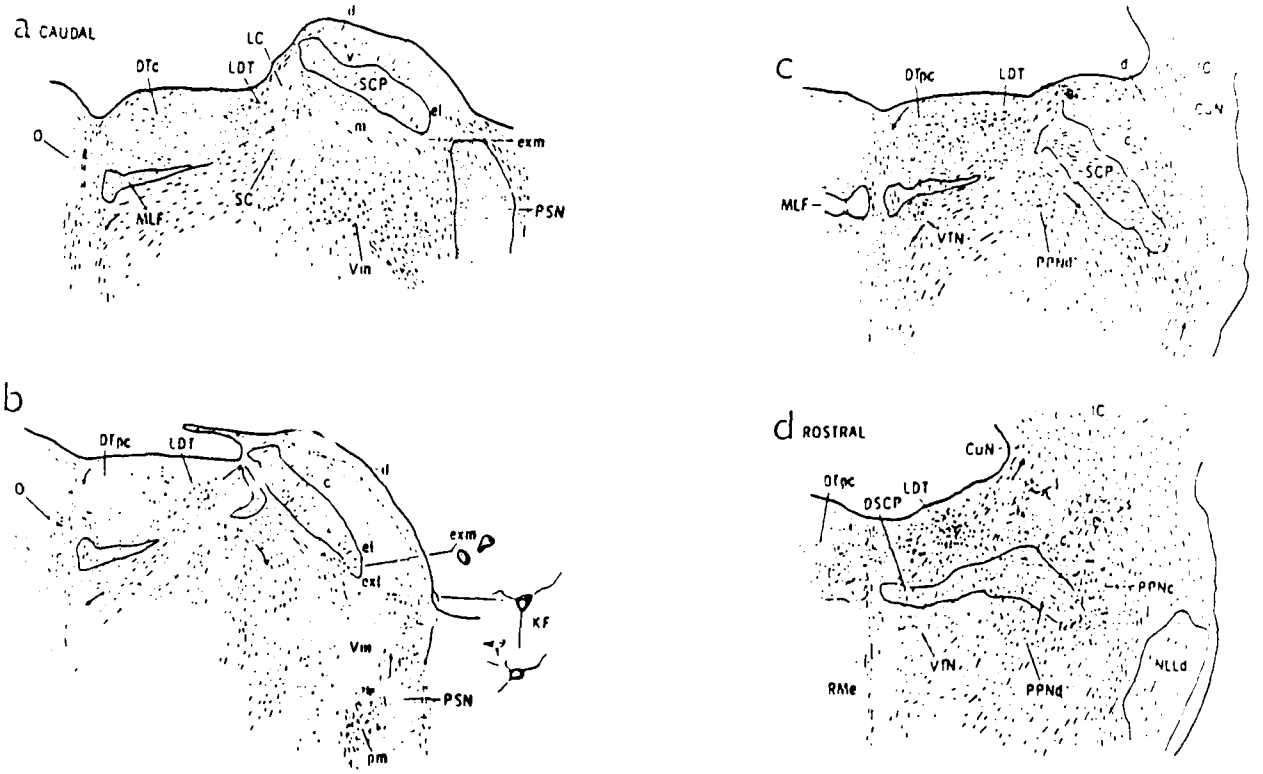


Figure 4. Camera lucida drawings focusing on the substructural organization of cholinergic processes (resembling terminal fields) in the parabrachial complex (PBC) and their topographic relationships to neighboring nuclei. Punctate varicosities labeled for ChAT outline portions of the dorsal-, central-, superior- and internal-lateral subnuclei and the extreme-medial and medial nuclei.

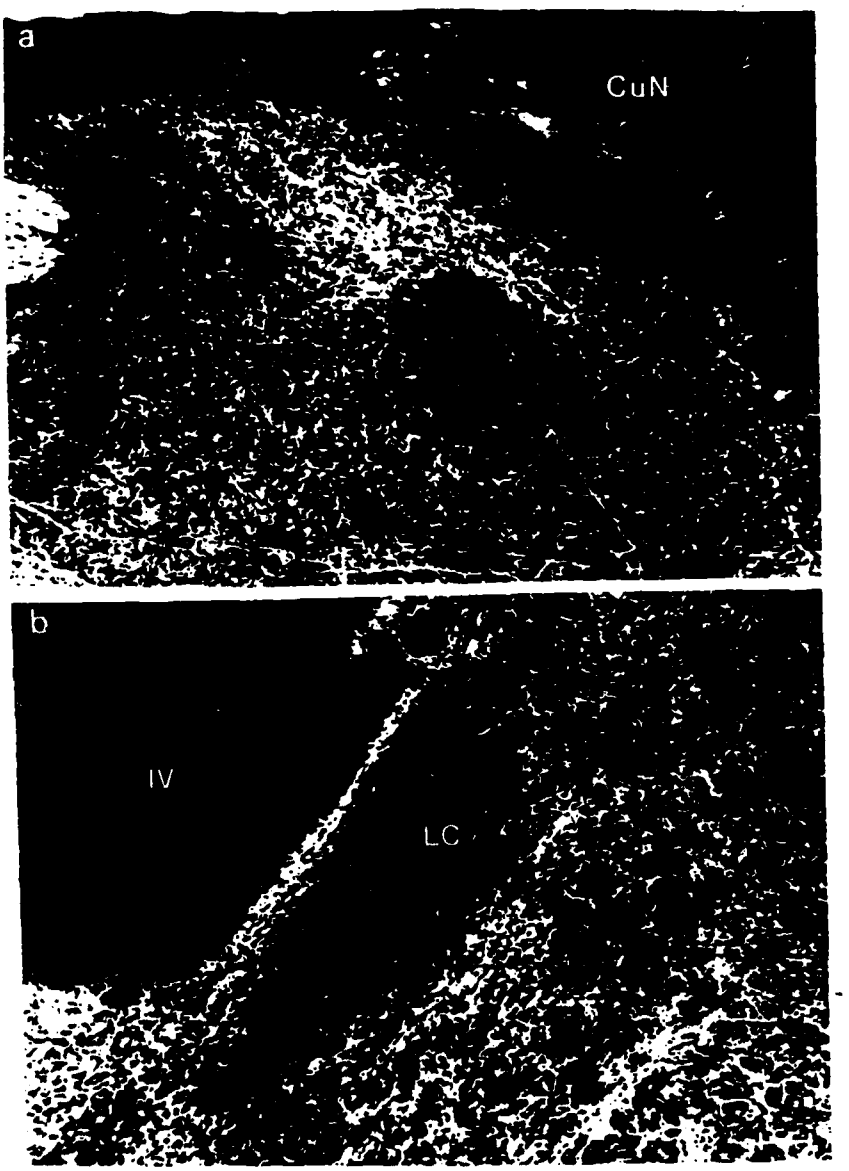


Figure 5. Darkfield photomicrographs demonstrating clusters of punctate varicosities in the dorsolateral pons. In (**a**), note the terminal-like cluster in the dorsal-lateral subnucleus of the parabrachial complex (PBC). In (**b**), note the virtual absence of punctate varicosities in the locus ceruleus (LC). Bar = 120 μm.

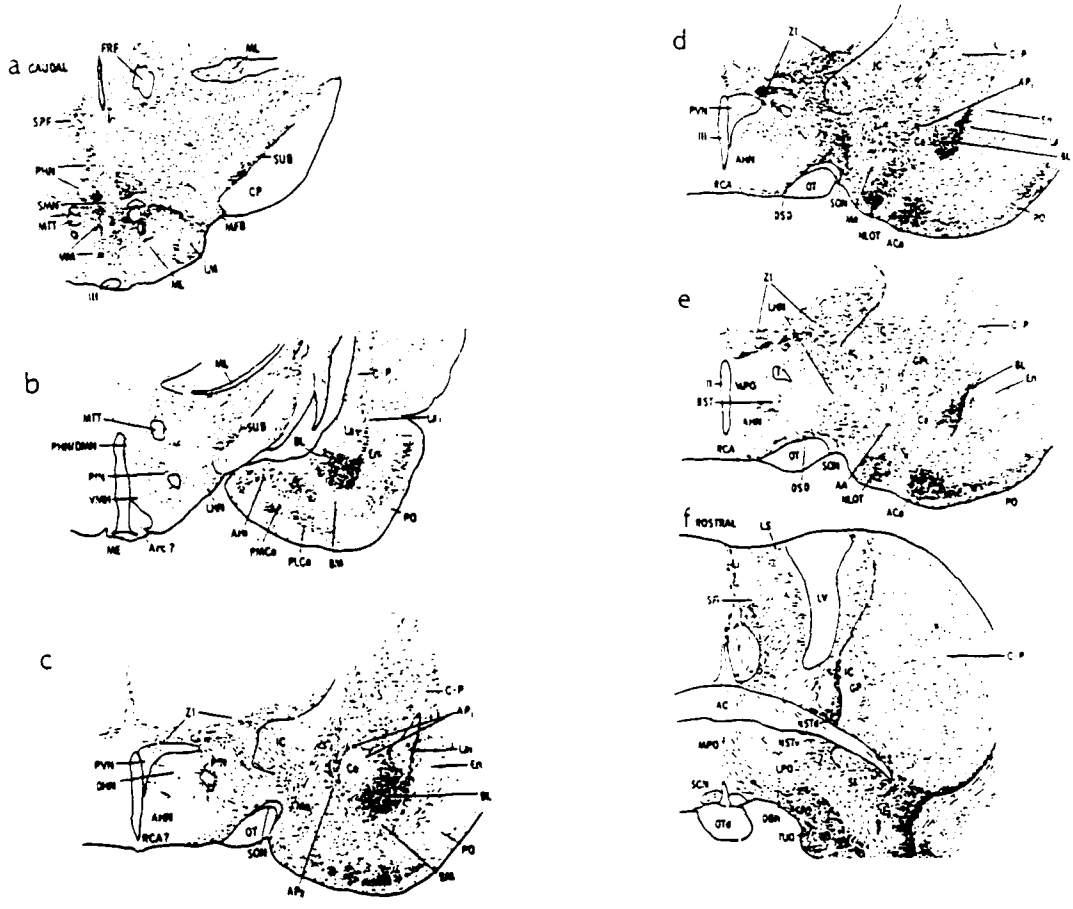


Figure 6. Camera lucida drawings of ChAT-immunoreactive processes in the forebrain. In the hypothalamus, punctate varicosities (terminal fields) outlined nuclei known to play a role in cholinergic regulation of arterial blood pressure and heart rate (e.g. the posterior and mammillary hypothalamic nuclei in subparafascicular, dorsal, perifornical and lateral hypothalamic nuclei, the bed nucleus of the stria terminalis and amygdala.

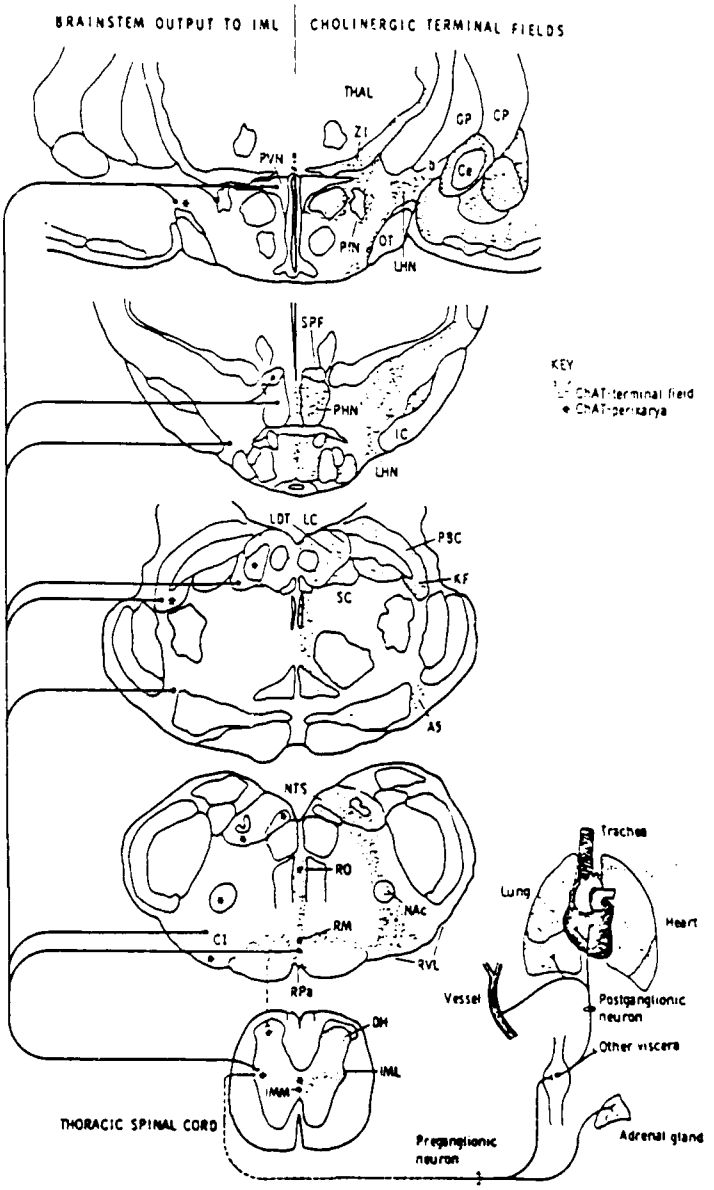


Figure 7. Schematic diagram summarizing the potential neuroanatomical substrates of cholinergic autonomic regulation. Cholinergic terminal fields (*right side*) overlap nuclear subgroups which contain catecholamine- and neuropeptide-synthesizing neurons and project directly to the intermediolateral cell column (IML; *left side*) or cervico-thoracic respiratory lower motor neurons influencing breathing (*not illustrated*).

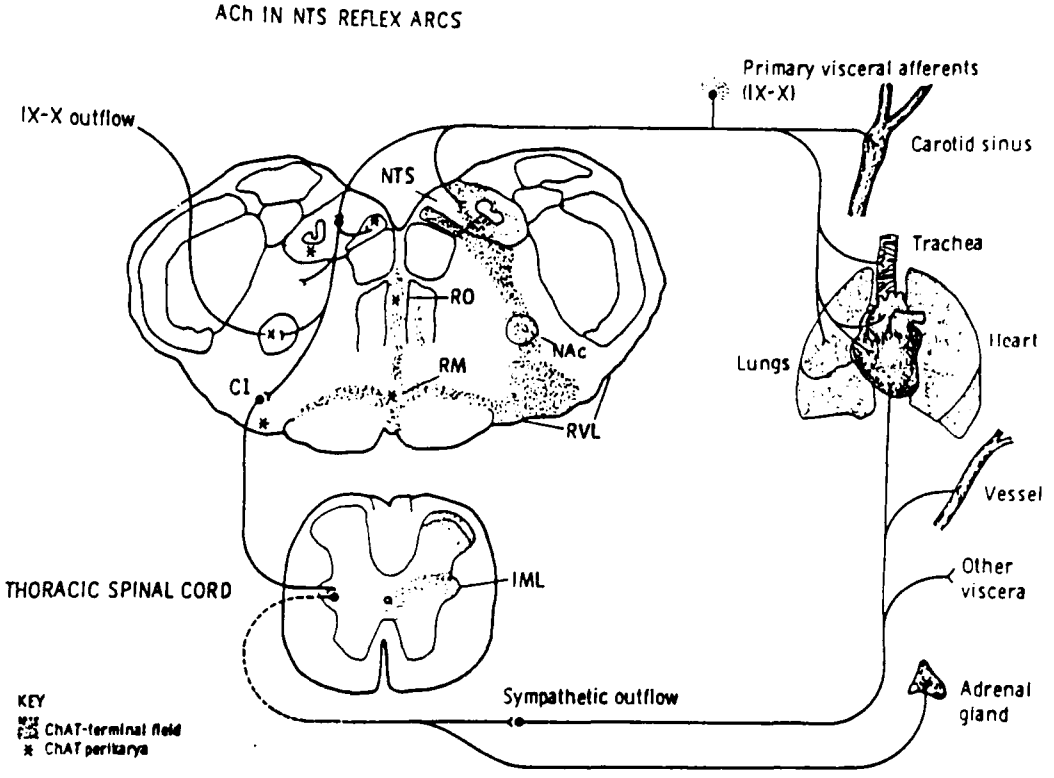


Figure 8. Summary diagram suggesting a role for acetylcholine in NTS-reflex control of cardiopulmonary function. Cholinergic terminal fields overlap subnuclei of the NTS receiving primary cardiopulmonary afferents and efferent projection sites of the NTS including the nucleus ambiguus and the rostral ventrolateral reticular nucleus (RVL).

DISTRIBUTION LIST

1 copy	Commander US Army Medical Research and Development Command ATTN: SGRD-RMI-S Fort Detrick, Frederick, Maryland 21701-5012
5 copies	Commander US Army Medical Research and Development Command ATTN: SGRD-PLE Fort Detrick, Frederick, Maryland 21701-5012
2 copies	Defense Technical Information Center (DTIC) ATTN: DTIC-DDAC Cameron Station Alexandria, VA 22304-6145
1 copy	Dean School of Medicine Uniformed Services University of the Health Sciences 4301 Jones Bridge Road Bethesda, MD 20814-4799
1 copy	Commandant Academy of Health Sciences, US Army ATTN: AHS-CDM Fort Sam Houston, TX 78234-6100